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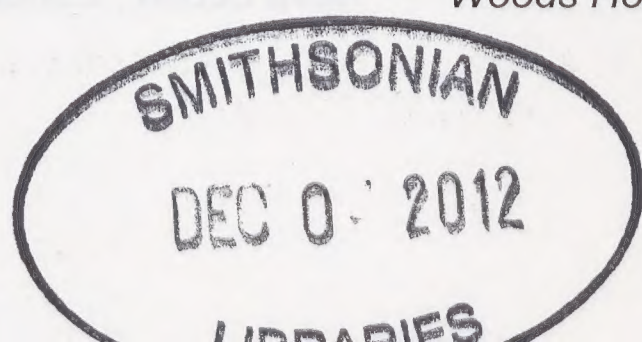
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ANNOUNCEMENT**The J Frances Allen Institute of Malacology
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The Board of Directors of the Institute of Malacology, publisher of the academic journal *Malacologia*, is pleased to announce the creation of the J Frances Allen Institute of Malacology Outstanding Publication Award. This cash award of \$500 will be presented for an outstanding paper published in *Malacologia* by a student, with the timing dependent on the superior quality of the student paper as judged by a committee of the IM Board. There is no formal application or deadline. A student submitting a manuscript must be sole or senior author of the paper, and should indicate that the paper being submitted stems from Master's or Ph.D. research to confirm his or her eligibility for the award. The Allen Publication Award was established in 2011 to honor J Frances "Jady" Allen, who provided indispensable help and guidance in establishing the journal *Malacologia*, first published in 1962.

SHELL SHAPE DIFFERENCES BETWEEN TWO *PANOPEA* SPECIES AND PHENOTYPIC VARIATION AMONG *P. GLOBOSA* AT DIFFERENT SITES USING TWO GEOMETRIC MORPHOMETRICS APPROACHES

Ignacio Leyva-Valencia¹, Sergio Ticul Álvarez-Castañeda¹, Daniel B. Lluch-Cota¹, Sergio González-Peláez¹, Sergio Pérez-Valencia^{1, 4}, Brent Vadopalas², Saul Ramírez-Pérez³ & Pedro Cruz-Hernández^{1*}

ABSTRACT

Most previous studies identifying *Panopea generosa* and *P. globosa* have used non-rigorous visual methods as well as older shell measurement techniques. Newer mathematical methods based on shell shape variation allow for more accurate identification of clam species, as well as modeling of phenotypic differences due to environmental effects in populations in different sites. Interspecific shell morphology for two Mexican geoduck clam species was analyzed from a total of five sites off both coasts of the Baja California peninsula. In addition, intraspecific analyses of shell morphology were conducted for one of the species, *P. globosa*, at four sites along its reported distribution. Two approaches were employed for the analyses: a novel approach based on radiating lines to characterize shell outlines, and a more traditional approach using internal shell landmarks. In general, the novel approach afforded greater fidelity in distinguishing inter- and intraspecific variation. Our results from both methods agree with original species descriptions, and showed that Bahía Magdalena geoducks are *P. globosa*, thus revealing a wider distribution than previous reports for this species. The outline and internal scars were highly discriminant between the two species. Shell shape of *P. generosa* was also less variable than that of *P. globosa*. Intraspecific analyses of *P. globosa* shell shape suggest an adaptive or phenotypic response to environmental conditions at each site. Our results may also be indicative of reproductive isolation between Pacific *P. globosa* at Bahía Magdalena and conspecifics in the Gulf of California.

Key words: *Panopea*, geoduck, shape differences, geometric morphometrics.

INTRODUCTION

Mollusks of the genus *Panopea* include among the largest of all deep-burrowing bivalves, with congeners occurring worldwide in intertidal and subtidal regions in the Pacific and Atlantic Oceans, the Mediterranean Sea, as well as off the coasts of Australia and New Zealand (Yonge, 1971). Two *Panopea* species, *P. generosa* (Gould, 1850) and *P. globosa* (Dall, 1898), commonly called the “geoduck clam”, occur in coastal waters of the Baja California Peninsula in both the Pacific Ocean and the Gulf of California.

Some authors report *P. generosa* distributions from south of Alaska to the Baja California pen-

insula (Weymouth, 1921; Fitch, 1952; Moore, 1968; Morris et al., 1980; Goodwin & Peace, 1989; Coan et al., 2000), whereas others include Baja California Sur (SAGARPA, 2007) and Gulf of California (DFO, 2000; Moore, 2001; Feldman et al., 2004; Deméré & Scott, 2006) within its distribution range. In contrast, the distribution of *P. globosa* has been reported as limited to the northern Gulf of California (Keen, 1971; Coan et al., 2000; Aragón-Noriega et al., 2007; Rocha-Olivares et al., 2010), although specimens were recently reported from the central eastern side of the Gulf of California off the mainland coast of Mexico near Guaymas, Sonora (Aragón-Noriega et

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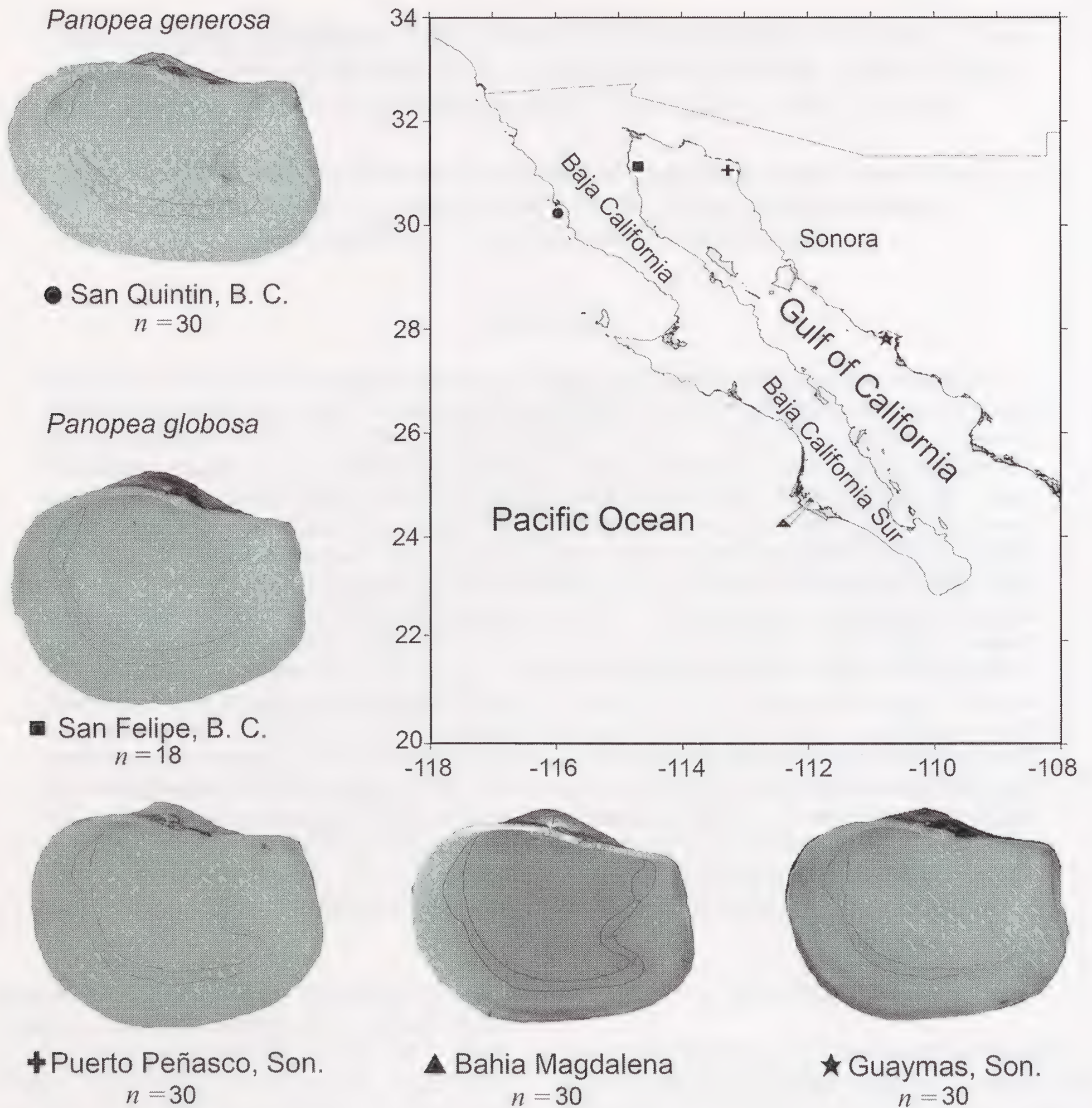


FIG. 1. Sampling sites of two *Panopea* species in northwest Mexico.

al., 2007), as well as the Pacific side of the Baja California peninsula in coastal waters of Bahía Magdalena (Fig. 1; González-Peláez, Centro de Investigaciones Biológicas del Noroeste, México 2010, pers. comm.).

Misidentification of *Panopea* species, on the other hand, remains a problem since morphometric plasticity is known to occur in the genus. For example, one case that is well documented is that of *Panopea generosa*, which had been mistakenly synonymized with the extinct *P. abrupta* for almost 25 years (Vadopalas et al., 2010).

Moreover, identification can be difficult because bivalve shell morphology is influenced

by such environmental factors as temperature, tidal excursion, wave exposure, water currents and sediment type (Costa et al., 2008a). The marine environment along the peninsula and the Gulf of California is highly variable, with seasonal circulation patterns (Zaytsev et al., 2003; Kessler, 2006; Lavín & Marinone, 2003) and differences in bathymetry (Bray, 1988; Lluch-Cota et al., 2007) that may influence regional morphological changes in metapopulations (Helenes & Carreño, 1999; Bizzarro, 2008; Álvarez et al., 2009).

The relationship between local environmental conditions and shell morphology in *Panopea*

TABLE 1. Geoduck (*Panopea* spp.) sampling sites and number of specimens used for interspecific and intraspecific geometric morphometric analysis.

Locality	Abbreviation	Location	Specimens
San Quintín	SQ	30°23'N, 115°57'W	30
San Felipe	SF	31°01'N, 114°49'W	18
Puerto Peñasco	PP	31°18'N, 113°33'W	30
Guaymas	GU	27°56'N, 110°51'W	30
Bahía Magdalena	BM	24°39'N, 112°03'W	30

are unknown. Given the propensity for variability in valve shape due to regional environmental variation (Fyfe, 1984), characterizing the variation in shell morphology between and within *Panopea* species could allow for more accurate estimates of population distributions. Shell morphology and structural traits are the principal characters used in traditional taxonomy of bivalves, including *Panopea generosa* and *P. globosa*. The shell of adult *P. generosa* is heavy, ponderous, subquadrate, subequilateral and the valves gape widely (Coan et al., 2000; Vadopalas et al., 2010). By comparison, the shell of adult *P. globosa* is more inflated, expanded and rounded in the pedal region, with a narrower posterior end and non-parallel opposite margins (Dall, 1898). Fyfe (1984) described the shell morphology of *P. generosa* as highly variable and taxonomically difficult to identify using traditional morphometric data (length, depth, and width). Thus, morphological differences between congeners of *Panopea* and its conspecifics are often difficult to identify by inexperienced observers.

Because linear measurements can be fairly ambiguous (Zelditch et al., 2004), researchers have explored geometric methods as an alternative for quantifying and analyzing morphological variation. Some of the more promising methods include the use of points that fall at defined intervals along a curve between two distinct landmarks; Marko & Jackson (2001) termed these points semi-landmarks.

Geometric morphometrics in other taxa have been shown to be powerful tools for solving complex species-level identification problems (Matias et al., 2001; Gumiel et al., 2003; Shipunov & Bateman, 2005). Relatively few studies have applied these techniques to mollusks. Some focused on general morphology (Ubukata, 2003; Anderson & Roopnarine, 2005; Carvajal et al., 2005, 2006; Roopnarine

et al., 2008), while others used geometric morphometrics to compared external shapes among conspecific populations (Kwon et al., 1999; Costa et al., 2008b) or phenotypic stocks (Márquez et al., 2010).

To help resolve the difficulty in species identification, which is affected by shell shape variation, we used two methods of geometric morphometric analyses to characterize shell shape differences in *Panopea* spp. from five sites on the Baja California peninsula. The first method is Procrustes superimposition which, to our knowledge, has not yet been used with bivalves. This newer method involves generating radiating lines, which fan out from a centroid and is referred to herein as "Fan-Based." The second method is based on anatomical landmarks of the inner shell.

MATERIALS AND METHODS

Samples and Sites

For taxonomic identification, we used the original descriptions (Conrad, 1849; Gould, 1850; Dall, 1898), recent identification keys (Keen et al., 1971; Coan et al., 2000) and the direct help of Paul Valentich-Scott (Curator of Malacology, Santa Barbara Museum of Natural History). Using SCUBA, divers collected adult *P. generosa* specimens from San Quintín (n = 30) and a total of 108 adult *P. globosa* specimens from San Felipe (n = 18), Puerto Peñasco (n = 30), Guaymas (n = 30) and Bahía Magdalena (n = 30; Table 1; Fig. 1).

Imaging and Measurement

The principles of the FB method are well described elsewhere (e.g., Sheets et al., 2006a; Rohlf, 1990; Lawing & Polly, 2009). For this

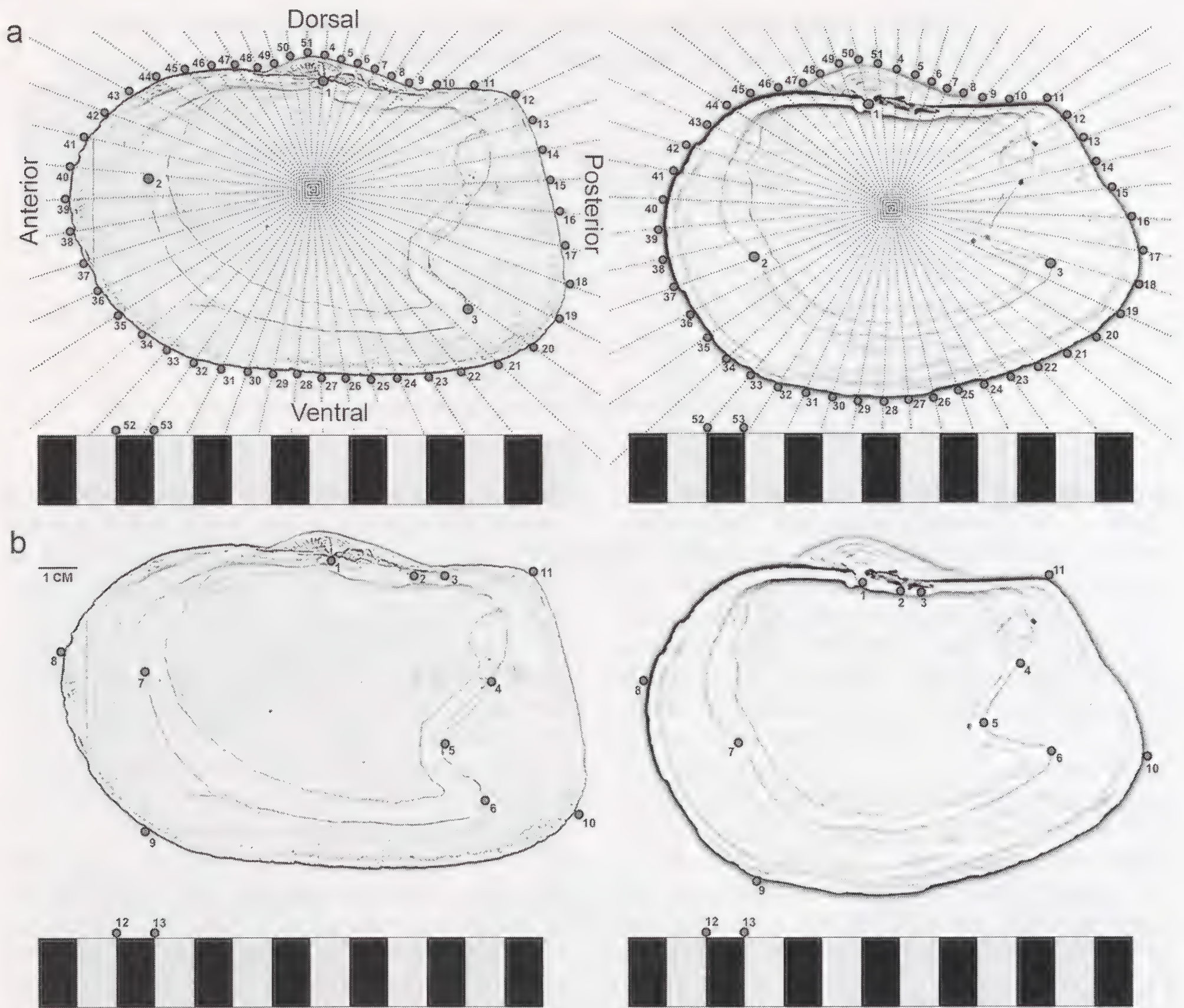


FIG. 2. Digitized landmarks and coordinates located for *Panopea generosa* (left) and *P. globosa* (right). Where (a) is the fan-based method showing the intercept between the valve contour and the drawing alignment fan (48 equal quadrants), and (b) is the internal scar method showing landmarks 1–3 which describe nymph shape while landmarks 4–6 describe the deep pallial sinus.

method, the set of points was defined *a priori* by an arbitrary rule: rays at equal angular intervals were used as guides to digitize semi-landmarks, with the points to be digitized located at the intersection of all radii of a circle and the outline curve (Sheets et al., 2006a; Rohlf, 1990; Lawing & Polly, 2009). As employed here, the FB method involved generating three anatomical landmarks and two-dimensional Cartesian coordinates of 48 semi-landmarks (Fig. 2a) using Makefan6 (Sheets et al., 2006b). We used these landmarks to align shapes via Procrustes superimposition. We used anatomical scars for landmarks 1, 2, and 3 (Fig. 2a) (umbo, antero-ventral adductor muscle, and postero-ventral pallial sinus) to determine centroid size, semi-landmarks 4 to 51 to analyze curves and

shell shape variation (semi-landmarks 4–11 and 45–51 to define the dorsal region; 12–20 to define the posterior end; 21–33 to delineate the ventral region, and 34–44 to define the anterior end). We used marks 52 and 53 as a reference scale (tpsDig 2.12, Rohlf, 2008). We used Semiland v6 software (Sheets et al., 2006b) to conduct FB analyses.

The ILB method analyzed the interior shape of each specimen using 11 landmarks (Fig. 2b). We used landmarks 1–3 to analyze nymph shape; 4–6 to analyze pallial sinus depth; landmark 7 to characterize the antero-ventral adductor scar and landmarks 8–11 to define outline curves. As above, we used landmarks 12 and 13 as a reference scale (Fig. 2b; tpsDig 2.12, Rohlf, 2008). We translated the interior

TABLE 2. Variance explained by the first two principal components between *Panopea generosa* and *P. globosa* and within *P. globosa* groups.

	Group	PC I (in %)	PC II (in %)	Overall (in %)
Outline				
Interspecific	<i>P. generosa</i>	52	19	71
	<i>P. globosa</i>	36	30	66
Intraspecific	SF	48	15	53
	PP	35	22	57
	GU	41	25	66
	BM	43	28	71
Internal landmarks				
Interspecific	<i>P. generosa</i>	33	25	58
	<i>P. globosa</i>	28	17	45
Intraspecific	SF	34	14	48
	PP	31	19	50
	GU	35	16	51
	BM	33	21	54

landmark coordinates to align the centroids of each individual. To minimize least-squares differences between landmark alignments, we used CoordGen6f (Sheets et al., 2006b) to rotate and scale centroid size to unity, producing Procrustes shape coordinates and consensus shapes for each group.

Analyses of Interspecific and Intraspecific Variation

To determine differences in outline (FB method) and internal landmarks (ILB method) between *P. generosa* and *P. globosa*, we used CVAGen6m (Sheets et al., 2006b) to perform canonical variates analysis and multivariate analysis of variance (CVA-MANOVA). To assess within-group shape variation, we used PCAGen6n software (Sheets et al., 2006b) to perform principal component analysis (PCA). To determine which canonical variates (CV) were effective discriminators, we employed Bartlett's test based on Wilk's lambda (Λ), which is the within-groups sum of squares divided by the total sum of squares. A value of Λ near 0 (zero) indicates high discrimination between groups (Zelditch et al., 2004).

We assessed the statistical significance of shape differences using Goodall's F-test. We calculated the full and partial Procrustes distances between means (DBM) of two

groups and bootstrapped to estimate the variance of this distance using TwoGroup6A software (Sheets et al., 2006b). We employed Mahalanobis distances (Mahalanobis, 1936) to determine the probability that the distance between an individual and the mean of the group was larger than expected under the null model of random variation around the mean of each group (Zelditch et al., 2004).

To visualize the shapes, we used thin-plate splines of the first two principal components. Thin plate splines provide graphical representations of the deformation of a square grid based on the difference in position of individual landmarks and displacement of the vectors relative to a grand mean consensus form (Bookstein, 1991).

RESULTS

Interspecific Differences

Fan-Based Method: CVA-MANOVA analysis of the FB method revealed a single CV with significant differences between species (Bartlett's test, Wilk's $\Lambda = 0.0836$; $p < 0.01$; Fig. 3a). Based on Mahalanobis distances, all specimens (*Panopea generosa*, $n = 30$ and *P. globosa*, $n = 108$) were correctly assigned to species. The partial Procrustes DBM between the two spe-

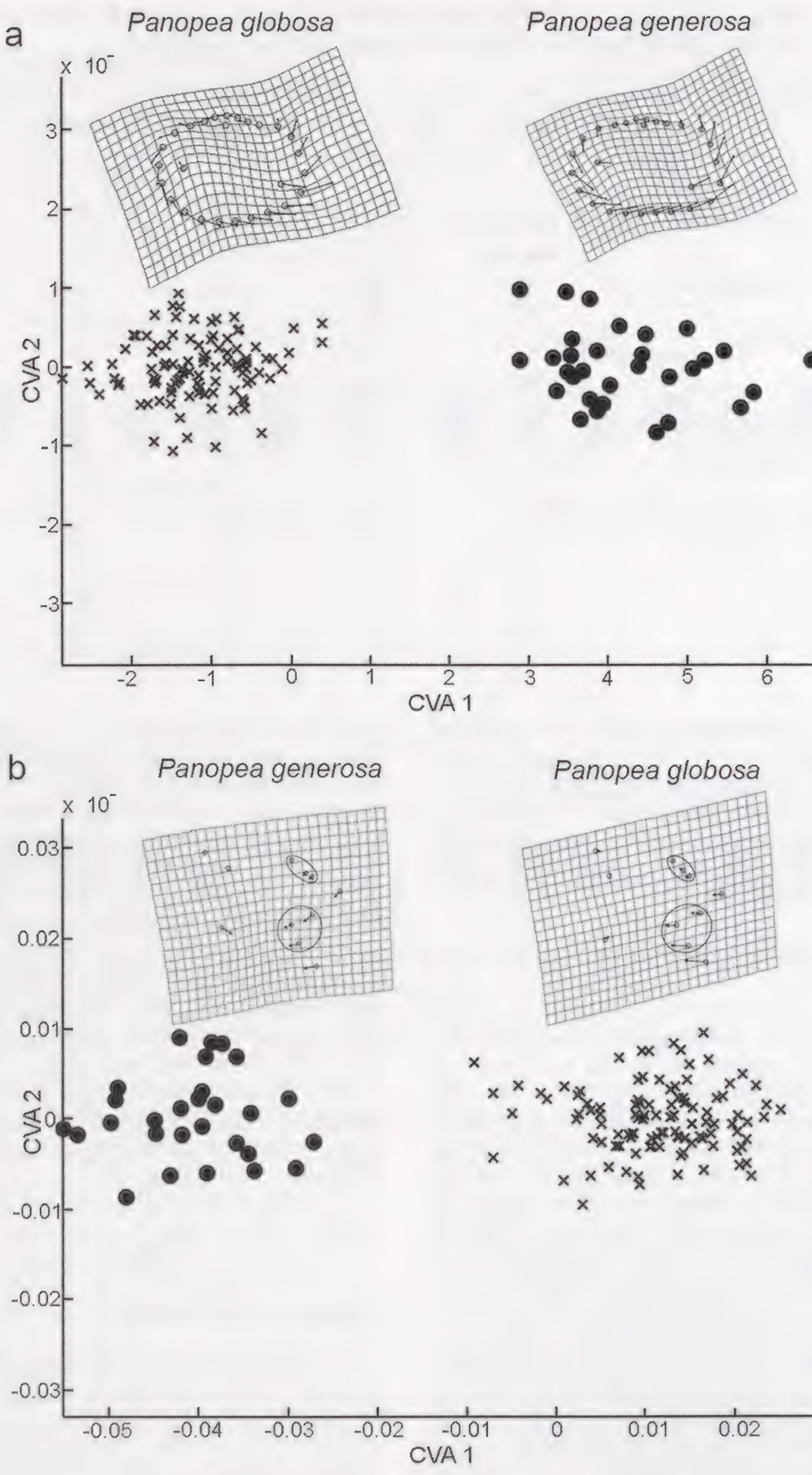


FIG. 3. Plot of interspecific canonical analyses of sets of variables and thin-plate spline with vectors of outline (a) and internal scars (b), nymph and pallial sinus, oval and circle respectively.

TABLE 3. Goodall's F-test and distances between means (DBM) of outline and internal landmarks in *Panopea globosa*. BM = Bahía Magdalena; GU = Guaymas; SF = San Felipe; PP = Puerto Peñasco. All comparisons were significant ($P < 0.01$).

	Comparison	F	DBM
Outline	BM vs GU	16.08	0.0475
	BM vs SF	6.25	0.0344
	BM vs PP	25.45	0.0589
	Gu vs SF	12.78	0.0440
	Guvs PP	14.94	0.0411
	SF vs PP	8.13	0.0342
Internal landmarks	BM vs GU	6.65	0.0540
	BM vs SF	6.35	0.0612
	BM vs PP	11.46	0.0702
	GU vs SF	7.90	0.0636
	GU vs PP	6.96	0.0517
	SF vs PP	2.69	0.0366

cies was significant (DBM = 0.065, Goodall's F-test with 900 bootstraps, $F = 43.8$, $p = 0$). The first two principal components (PC) accounted for 71% and 66% of the total variance in *P. generosa* and *P. globosa*, respectively (Table 2). Note that the loadings of all other PCs were less than 1%; they are not shown in Table 2, as they contribute little to the variance. In contrast, PC1 and PC2 fan rays contribute to most of the variance for the two species.

Inner Landmark-Based Method: CVA-MANOVA analysis of the ILB results revealed a single CV with significant differences between species (Bartlett's test, Wilk's $\Lambda = 0.0987$, $p < 0.01$; Fig. 3b). All *Panopea generosa* and *P. globosa* specimens were correctly assigned to species based on Mahalanobis distances. The partial Procrustes DBM of internal landmarks was significant (DBM = 0.0982, Goodall's F-test with 900 bootstraps, $F = 32.72$; $p = 0$). The first two PCs accounted for 58 and 45% of the total variance in *P. generosa* and *P. globosa*, respectively (Table 2).

When we compare the FB and ILB results, the thin plate splines of the first two PCs obtained by Procrustes superimposition of centroid size reveal easily perceivable shape differences in both the deformation grids and vector displacement (Fig. 3a, b).

TABLE 4. Intraspecific individual assigned with the Mahalanobis distance approach among groups of *Panopea globosa*. BM = Bahía Magdalena; GU = Guaymas; SF = San Felipe; PP = Puerto Peñasco.

		BM	GU	BM	GU
Outline	BM	30	0	0	0
	GU	2	28	0	0
	SF	0	0	18	0
	PP	0	1	0	29
Internal landmarks	BM	28	1	0	1
	GU	2	27	0	1
	SF	0	1	10	7
	PP	1	2	9	18

Intraspecific Differences

Fan-Based Method: Using the FB approach we observed three CVs of outline shape variation among *P. globosa* groups with CVA-MANOVA (Fig. 4a). Axis 1 (Wilk's $\Lambda = 0.0159$, $p < 0.01$) separated the Pacific group (Bahía Magdalena) from the Gulf of California groups (Guaymas, San Felipe, and Puerto Peñasco); Axis 2 (Wilk's $\Lambda = 0.0965$, $p < 0.01$) separated the southern Gulf group (Guaymas) from the northern Gulf groups (San Felipe and Puerto Peñasco); Axis 3 (Wilk's $\Lambda = 0.3620$, $p < 0.01$) placed San Felipe and Puerto Peñasco in the same group. Mahalanobis distances correctly assigned 100% of the specimens to the Bahía Magdalena and San Felipe groups. In contrast, correct allocation was slightly lower for the localities of Guaymas and Puerto Peñasco (93 and 97%, respectively). The first two PCs account for a high percentage of the total variance (58–71%) at all sites (Table 2). Goodall's F-test values and the partial Procrustes DBM among *P. globosa* groups outlines were significant (Table 3).

Inner Landmark-Based Method: When comparing shape variation among internal landmarks using the ILB approach in the same groups (Fig. 4b), we obtained two CVs: Axis 1 (Wilk's $\Lambda = 0.0800$, $p < 0.01$) discriminated the Pacific group (Bahía Magdalena) from the Gulf groups (Guaymas, San Felipe, and Puerto Peñasco), and Axis 2 (Wilk's $\Lambda = 0.3396$, $p <$

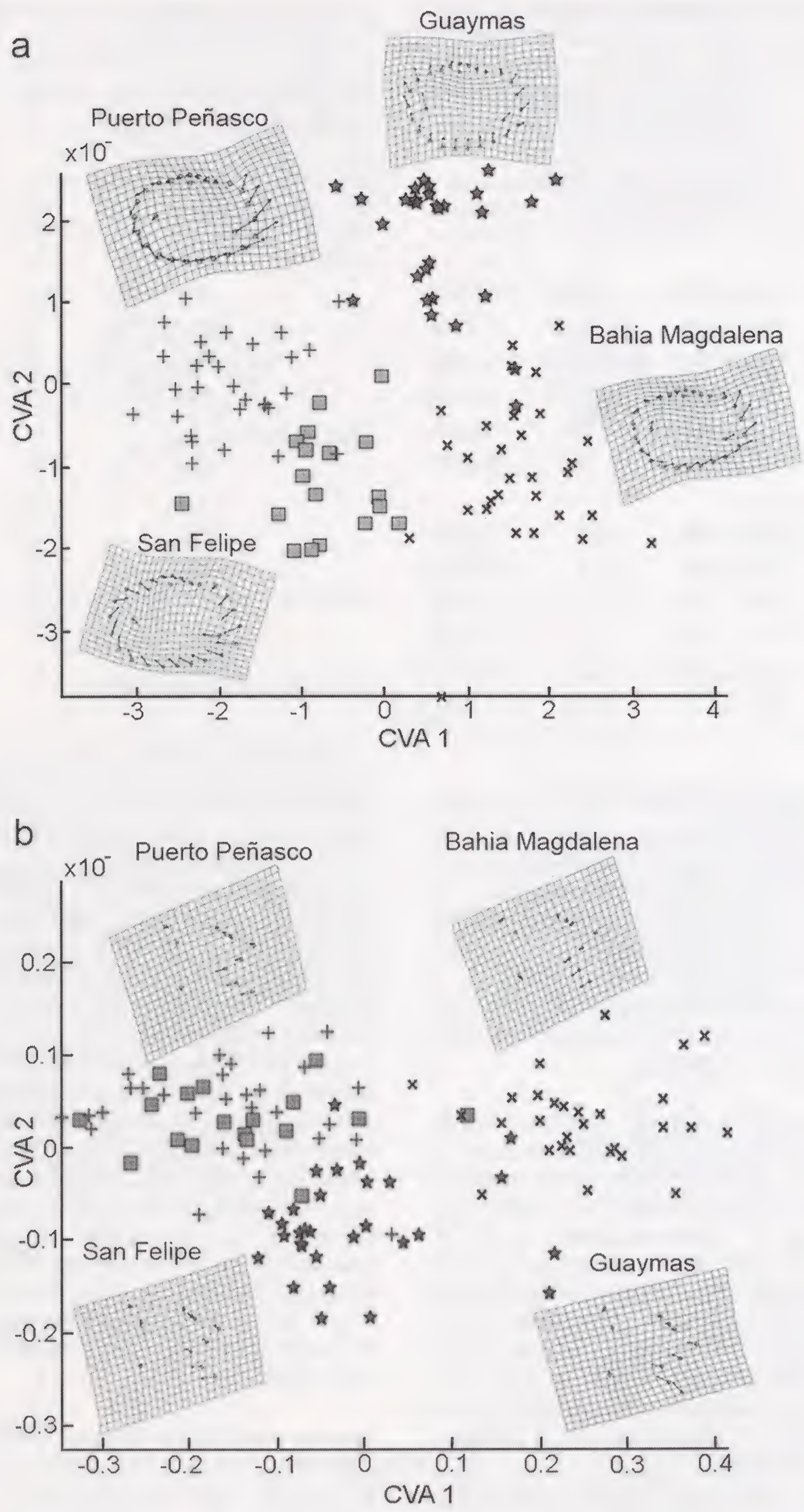


FIG. 4. Intraspecific canonical analyses of sets of variables plot of shell shape variation of *Panopea globosa* collections in northwest Mexico. Outline (a), internal scars (b).

0.01) included all Gulf groups. Group assignment with Mahalanobis distances revealed some incorrect assignments of northern Gulf geoducks: 37% of Puerto Peñasco specimens were grouped incorrectly with San Felipe, while 45% of the samples of San Felipe were incorrectly grouped with Puerto Peñasco. In contrast, > 90% of Bahía Magdalena and Guaymas specimens were correctly assigned (Table 4).

With these landmarks, the first two PCs accounted for 50–54% of the total variance (Table 2). Goodall's F-test values and DBM were also significant (Table 3). Thin plate splines revealed the greatest differences in vector length and displacement of landmarks 4–7 and 10 between Pacific *P. globosa* and the three Gulf groups (Fig. 4b).

DISCUSSION

In this study we used two methods to analyze interspecific and intraspecific phenotypic variation: a new proposed FB method, for external contours of the shell, as well as the more traditional ILB method for mapping the interior of the shells for geometric morphology analyses to determine interspecific shell shape differences in Mexican geoducks. Our results clearly delimited two groups that correspond to the two species, *Panopea generosa* and *P. globosa*, in agreement with the original descriptions (Gould, 1850; Dall, 1898). The specimens from Bahía Magdalena (Pacific coast) grouped with *P. globosa*, corroborating the traditional taxonomical procedure. The results indicate a much wider distribution for *P. globosa* than only the northern Gulf of California as previously reported (Coan et al., 2000; Briggs, 1974; Brusca, 1980; Hastings, 2000; Calderón-Aguilera et al., 2010; Rocha-Olivares et al., 2010).

Interspecific Shell Shape Variation

Prior studies using traditional morphometric analyses have shown notable shape differences between *Panopea generosa* and *P. globosa* (Rocha-Olivares et al., 2010). Using three linear measurements, these authors found that *P. generosa* had a more homogeneous shell length compared to *P. globosa* and they successfully discriminated the two species. The present study corroborates this finding and increases the resolution of inter-specific morphological shell variation.

The ILB method has been used to determine shell shape variation in other bivalves. For example, Innes & Bates (1999) determined contour differences between *Mytilus edulis* and *M. trossulus* (Wilk's $\Lambda = 0.625$), whereas Roopnarine & Vermeij (2000) reported Wilk's $\Lambda = 0.25$ for internal shape variation between *Chione cancellata* and *C. elevata*. In contrast, in our study Bartlett's test was highly discriminant between the two *Panopea* species (Wilk's $\Lambda = 0.08$ and 0.09 for FB and ILB, respectively).

The consensus outline shape of *Panopea globosa* is more inflated, with a narrower posterior end compared to *P. generosa*. The umbo in *P. globosa* is near the posterior end, while more centrally located in *P. generosa*. The off-center placement of the umbo in *P. globosa* may be related to an anatomical adaptation which aids in maintaining valve gape due the more inflated shell; Savazzi (1987) discusses the hypothesized relationship between shell inflation and umbo displacement, and Ubukata (2003) observed in pteroids a tendency of shell extension in the antero-ventral direction.

In our study, variation in the inner shell indicates that *Panopea globosa* has a shorter nymph and smaller ligament (landmarks 1–3), but a deeper pallial sinus than *P. generosa* (landmarks 4–6). Stanley (1970, 1979) and Lockwood (2004) describe the pallial sinus as the space where siphonate bivalves contract the siphon. Kondo (1987) suggested that the ratio of pallial sinus depth to shell length is closely correlated to the maximum burrowing depth. Although we found a deeper pallial sinus in *P. globosa* than in *P. generosa*, we did not measure syphon length or burrowing depth thus we are unable to corroborate Kondo's hypothesis.

The FB and ILB methods showed that the first two PCs in *Panopea generosa* accounted for more variability in shell shape than in *P. globosa* (Table 2). To fully corroborate these results in a future study, it will be necessary to examine specimens of *P. generosa* from other sites.

Panopea globosa Intraspecific Shell Shape Variation

Both the FB and ILB methods revealed that *P. globosa* of Bahía Magdalena are morphologically distinct from the three other localities of the Gulf of California. Nevertheless, the FB method more clearly distinguished three different groups, while the more traditional ILB method exhibited lower fidelity since it was only able to distinguish two groups.

Our results suggest a shell shape adaptation or phenotypic response to environmental conditions at each site. The intraspecific CVA analyses reveal significant differences among *Panopea globosa* sites, where Pacific *P. globosa* (Bahía Magdalena) have more elongated shells but a shorter pallial sinus than those in the Gulf of California. Moreover, within the Gulf, our analyses revealed that Guaymas geoducks are smaller and have a more inflated shell and deeper pallial sinus compared to those from San Felipe and Puerto Peñasco. Rocha-Olivares et al. (2010) also reported similar size differences between *P. globosa* from the upper and middle Gulf.

Shape differences might also be explained by genetic differences, as observed in pectinids from the Pacific and Gulf sides of the Baja California Peninsula (Cruz & Ibarra, 1997; Cruz et al., 1998), or by phenotypic plasticity noted in other bivalve populations subjected to different environmental conditions (Palmer et al., 2004; Krapivka et al., 2007; Costa et al., 2008a; Elliot et al., 2008; Sokolowski et al., 2008; Márquez et al., 2010). In addition, morphological polymorphisms may result from gene-environment interactions (Rawson & Hilbish, 1991; Trapani, 2003). The variation in *P. globosa* we observed suggests either phenotypic plasticity or local adaptation due to gene-environment interaction.

Shell shape differences between Bahía Magdalena and Gulf of California geoducks might be associated with temperate environmental conditions, in contrast to the northern Gulf, which is a warm-temperate region (Lluch-Belda et al., 2000; Calderón-Aguilera et al., 2010). On the other hand, the lower shape differentiation between San Felipe and Puerto Peñasco might be caused by gene flow due to geographical proximity and circulation patterns, since the anticyclonic circulation during November-April in the northern Gulf (Lavín & Marinone, 2003) exactly overlaps with the reproductive period for *P. globosa* (Calderón-Aguilera et al., 2010).

The notable shell shape differences of *Panopea globosa* in Guaymas may be due to drastically higher temperatures conditions during the summer months (Lluch-Cota & Arias, 2000; Green et al., 2005); the most abundant beds on this site are located in shallower waters (8–12 m, Aragón-Noriega et al., 2007, Rocha-Olivares et al., 2010) compared with the other studied localities (Bahía Magdalena 10–20 m, González-Peláez Centro de Investigaciones Biológicas del Noroeste, México 2010, pers.

comm.; San Felipe, Puerto Peñasco 10–25 m, Rocha-Olivares et al., 2010). In addition, primary productivity is lower in Guaymas since there are essentially no currents most of the year (Lluch-Cota & Arias, 2000; Green et al., 2005).

The novel FB method of contours was found to have higher resolution than the traditional ILB method for distinguishing intraspecific phenotypic differences among the three *Panopea globosa* groupings: (1) Bahía Magdalena; (2) San Felipe-Puerto Peñasco; and (3) Guaymas. Geometric morphometric analyses suggest the possibility of geographic and breeding isolation between *P. globosa* of Bahía Magdalena in the Pacific and their conspecifics from the Gulf of California. Genetic studies are underway to understand the evolutionary history of *P. generosa* and *P. globosa* and the genetic relationships among populations of *P. globosa*.

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OCCURRENCE OF THE INVASIVE SPECIES *POTAMOPYRGUS ANTIPODARUM* (PROSOBRANCHIA: HYDROBIIDAE) IN MINING SUBSIDENCE RESERVOIRS IN POLAND IN RELATION TO ENVIRONMENTAL FACTORS

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ABSTRACT

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843), is recognized as a successful invader of aquatic environments in Australia, Europe, Japan, North America and western Asia. To date, a long-term survey on the occurrence of *P. antipodarum* (including its initial dispersal, establishment and integration) both in mining subsidence reservoirs and an adjacent river in Poland has not been carried out. The objectives of this survey were to analyze the environmental factors that influence the occurrence of *P. antipodarum* in relation to mollusc communities in mining subsidence reservoirs affected by coal mine output in terms of initial dispersal and the establishment of self-sustaining populations in the new habitat, to analyze annual variations in the densities and shell height of *P. antipodarum* in relation to the different types of substratum, and to determine the number of embryos in the brood pouch per female at the integration stage (phase). During the years 1993–2008, seven mining subsidence reservoirs that have different water sources and a part of the Mleczna River were investigated.

From 1997 to the present, *P. antipodarum* has been eudominant in mollusc communities both in coal mining subsidence reservoirs, which receive water from the mine dewatering system and in the river. Based on a redundancy analysis (RDA), the conductivity of water and the concentration of nitrates were the parameters most associated with the distribution of mollusc species, including *P. antipodarum*. The relation between the species and these environmental variables was statistically significant.

These results do not suggest that the integration of *P. antipodarum* has negatively influenced the occurrence of other mollusc species either in the mining subsidence reservoirs or in the Mleczna River, probably due to smaller densities compared with a survey of the western United States, where *P. antipodarum* has a strong influence on aquatic ecosystems and restricts the occurrence of native macroinvertebrates. *Potamopyrgus antipodarum* has established itself in these mining subsidence reservoirs and is integrated into the local biota without any major negative effects (e.g., extirpations) on the communities being invaded.

Key words: *Potamopyrgus antipodarum*, alien species, RDA, environmental factors, shell height, embryos, mining subsidence reservoirs.

INTRODUCTION

One of the factors that influences the species balance in native aquatic fauna is the arrival of new species from other geographical areas. A new species may become integrated into the local biota without extinctions of native organisms or may have a range of effects on the extirpation of native species. The general process of an invasion can be divided into three basic stages: arrival (initial dispersal), establishment of self-sustaining populations

within the new habitat, and integration (Moyle & Light, 1996; Puth & Post, 2005) or to be more detailed, into five stages: from 0 (a donor region) to V (widespread and dominant) (Colautti & MacIsaac, 2004).

The New Zealand mud snail, *Potamopyrgus antipodarum* (Gray, 1843), is recognized as a successful invader of aquatic environments in Australia, Europe, Japan, North America (Ponder, 1988; Mitsuaki & Ryoji, 2004; Richards & Shinn, 2004) and western Asia (Naser & Son, 2009).

In Europe, *P. antipodarum* was recorded for the first time (initial dispersal) in England at Grays, Essex before 1852 (Kerney, 1999). It was probably introduced there by way of drinking water supplies on ships and then liberated into such estuarine areas as the River Thames during the washing or filling up of water barrels or tanks (i.e., transported out of its native range via long-distance transport vectors and then released within a new locality).

Potamopyrgus antipodarum has successfully colonized freshwater and brackish habitats in almost all of Europe: Great Britain (Fish & Fish, 1989; Kerney, 1999), the Iberian Peninsula (Sousa et al., 2007a; Múrria et al., 2008), France, Finland (Carlsson, 2000; Leppäkoski & Olenin, 2000), Sweden, Russia, Belarus (Karatayev et al., 2008), Lithuania, Latvia, the Ukraine (Con, 2006), Slovakia (Šteffek, 2000), the Czech Republic (Beran, 2002), Austria (Patzner, 1996), Switzerland (Baur & Ringeis, 2002), Hungary (Héra, 2005) and Greece (Radea et al., 2008).

In Poland, *P. antipodarum* was recorded for the first time in inland waters in its initial dispersal stage in 1933. At present, this invasive species occurs in brackish and freshwater habitats in many parts of Poland (Jackiewicz, 1973; Strzelec, 1993; Brzeziński & Kołodziejczyk, 2001). In Upper Silesia, southern Poland, *P. antipodarum* was recorded for the first time in a sand pit pond in 1986 (Strzelec & Krodkiewska, 1994) (stage II: introduction). Currently, *P. antipodarum* has inhabited most aquatic environments in Upper Silesia: rivers, streams and anthropogenic reservoirs (Strzelec, 1999a; Michalik-Kucharz et al., 2000; Lewin & Smoliński, 2006); this is the third (III) stage establishment.

Colonization and dispersal of *P. antipodarum* is enhanced by parthenogenesis (Wallace, 1979, 1992) and is euryhalinic. These factors could be major contributors to the rapid rate of the New Zealand mud snail's spread (establishment of self-sustaining populations within the new habitat) throughout Europe. Salinity is an important range limitation factor for most aquatic species compared to other ecological factors, such as food supply, competition, and predators. *Potamopyrgus antipodarum* is able to tolerate salinity of up to 28‰ (Costil et al., 2001) or even up to 30‰ (Paavola et al., 2005). *Potamopyrgus antipodarum* may be transferred by many local and long-distance vectors, such as birds, fish, insects, lymnaeid snails (when ingested by them, they can go through the

digestive system and remain alive) (Piechocki, 1999; Piechocki & Kaleta, 2001; Vinson, 2004; Bersine et al., 2008), fish stocking and angling (Loo et al., 2007); boats and ships (attached to their hulls), through the ballast water of commercial ships, within freshwater tanks and water pipes (Alonso & Castro-Díez, 2008).

Potamopyrgus antipodarum occurs more frequently at aquatic sites with multiple land uses in a catchment area compared to sites with low-impact human activities (Schreiber et al., 2003). A wide tolerance range to physical and chemical factors, the ability for active and passive dispersal, ovoviviparity, parthenogenesis and a high reproduction rate may explain the spread and integration success of *P. antipodarum*. For example, in the USA, *P. antipodarum* can produce up to six generations per year and reproduction appears to be the highest during the summer months (Vinson, 2004).

Some data have indicated that *P. antipodarum* influences the impoverishment of native fauna in reservoirs in industrial areas (Strzelec, 2005). At extremely high densities, *P. antipodarum* may compete for food and the space occupied by native non-molluscan fauna (Strayer, 1999) or native snails (Department of the Interior, U.S. Geological Survey, 2002).

Upper Silesia, southern Poland, is the most urbanized and industrialized region with a natural environment that has been transformed over two hundred years, deteriorated on a large scale and polluted mainly by hard coal mining or the metallurgical industry. This area constitutes one of the biggest coal basins in the world. It has been mined since the eighteenth century. Upper Silesia has no natural water bodies; only reservoirs of an anthropogenic origin, including mining subsidence reservoirs are common. Most of these as well watercourses are characterized by high levels of chlorides, sulphates, TDS, biogenic elements, conductivity (of even up to 20,000 $\mu\text{S}/\text{cm}$) or heavy metals both in the water and bottom sediments (Rzetała, 2008). Anthropogenic reservoirs, which are not so degraded, have provided refuges for many species of fauna and flora as well as rare and vulnerable mollusc species. Thus, they constitute important lentic habitats in Upper Silesia.

To date, a long-term survey on the occurrence of *P. antipodarum* (including its initial dispersal, establishment and integration) both in mining subsidence reservoirs and the river has not been carried out.

The objectives of the survey were to (1) analyze the environmental factors which influence

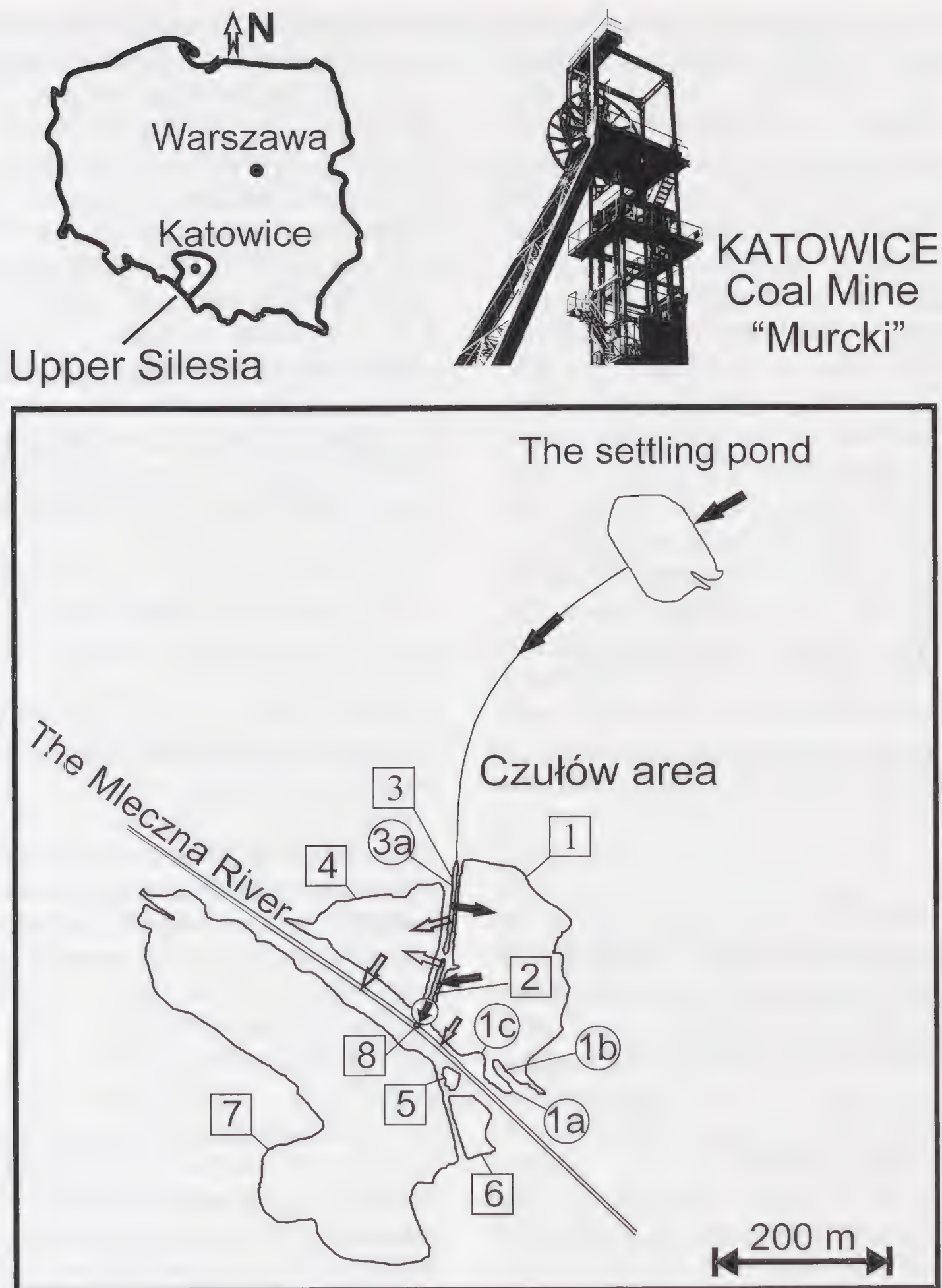


FIG. 1. Location of the study area. Sampling sites (biometrical analysis): 1, 2, 3, 4 = mining subsidence reservoirs with flowing water; 5, 6, 7 = mining subsidence reservoirs with non-flowing water; 1a = submerged leaves of *Glyceria maxima*; 1b = submerged leaves of *Typha latifolia*; 1c = muddy-stony bottom; 3a = muddy-sandy bottom; white arrow = water flow; black arrow = main water stream of the mining dewatering system.

the occurrence of the invasive species *P. antipodarum* in relation to mollusc communities in mining subsidence reservoirs affected by coal mine output in terms of initial dispersal and (2) the establishment of self-sustaining populations within the new habitat, (3) to analyze annual variations in densities and shell height of *P. antipodarum* in relation to the different types of substratum and (4) to determine the number of embryos in the brood pouch per female at the integration stage (phase).

MATERIALS AND METHODS

Study Area

The study was carried out from 1993–2008 in Czułów near Katowice, Upper Silesia, southern Poland. Seven mining subsidence reservoirs with different water sources were investigated. The part of the Mleczna River, which is connected with the mining subsidence reservoirs, was also investigated. The reservoirs were

developed from the operation of the "Murcki" coal mine in the 1970s. The mining subsidence reservoirs 1, 2, 3 and 4 (Fig. 1) are supplied with flowing water from surface waters and also with waters from the mine dewatering system. Mining water flows into reservoir 3 and then through reservoirs 1, 2, 4 and on into the Mleczna River. Mining subsidence reservoirs 5, 6, and 7 are reservoirs with non-flowing water. The surface areas of the reservoir amounts to: 100 (No. 5), 150 (No. 2), 240 (No. 3), 2,400 (No. 6) 3,200 (No. 4), 5,000 (No. 1) and 25,000 (No. 7) m², respectively. The average depth of the reservoirs ranges from 0.5 to 2.0 m. The reservoirs have now been reclaimed and the banks are partially concrete-lined and stabilized with waste rock and slag. The reservoirs, which are stocked with fish, are used by anglers and hunters. The macrophytes have been partially removed and the reservoirs' output has been regulated as part of the reservoir management process. This area provides a refuge for wild-fowl, birds of prey, amphibians and reptiles.

Methods

Samples of Molluscs and Water: Molluscs were sampled once each year from 1993–2008 using a 25 x 25 cm quadrat frame placed randomly in 16 locations (sampling area: 1 m²) within each of the reservoir beds and the river. Bottom sediments up to 5 cm, macrophytes and surface water were included within each sample. The collected material was sealed in plastic bags and plastic containers. The samples were then filtered using a 0.5 mm mesh sieve, sorted, and then the molluscs were preserved in 75% ethanol. The species of molluscs were identified according to Glöer & Meier-Brook (1998) and Glöer (2002). Mollusc density was estimated as the number of individuals per square meter. Water samples were collected from each sample site immediately prior to sampling and analyzed for the physical and chemical parameters of the water, that is, conductivity, pH, dissolved oxygen, nitrates, phosphates, total hardness, iron, chlorides and alkalinity. The total hardness was considered within following ranges: soft water from 90 to 180 mg CaCO₃/l, medium hard water above 180 to 270 mg CaCO₃/l, significantly hard above 270 to 360 mg CaCO₃/l, and hard water up to 450 mg CaCO₃/l (Hermanowicz et al., 1999). The significance of differences in the physical and chemical parameters among reservoirs with flowing water, non-flowing water and the river

was calculated by means of the Kruskal-Wallis one-way ANOVA test using the Statistica program version 8.0 (the value of parameters did not reveal a normal distribution and this justified the use of a non-parametric test).

For the biometrical analysis (e.g., the shell height measurement), every month, from June 2006 to June 2007, *P. antipodarum* were collected from a range of substrata in reservoir 1 (three sampling sites): sampling site (1a) submerged leaves of *Glyceria maxima* (Hartm.) Holmb., sampling site (1b) submerged leaves of *Typha latifolia* L., sampling site (1c) muddy-stony bottom and in reservoir 3 (one sampling site): sampling site (3a) muddy-sandy bottom, in order to sample the most microhabitats (Fig.1). Shell height was measured for 100 specimens of *P. antipodarum* randomly chosen from each of the sampling sites in each of the months. Each month, the number of embryos in the brood pouch was assessed for 30 specimens for which the shell height was larger than 3.5 mm.

Zoocenological Study: A zoocenological study of the mollusc communities was carried out to evaluate the dominance pattern of *P. antipodarum* in relation to other mollusc species:

(1) Dominance (D%)

$$D = n_a / n \times 100$$

where

n_a - the number of individuals of species a,

n - the total number of individuals in a sample.

The value of the dominance index D was divided into five classes according to Górny & Grüm (1981): eudominants > 10.0% of sample, dominants 5.1–10.0% of sample, subdominants 2.1–5.0% of sample, recedents 1.0–2.1% of sample, and subrecedents < 1.0% of sample.

(2) The Shannon-Wiener index (Cao et al., 1996; Mouillot & Lepretre, 1999)

$$H' = - \sum (P_i) (\log_2 P_i)$$

where

$P_i = N_i / N$ - the proportion of individuals belonging to species i.

The values of H' were calculated using the Multi-Variate Statistical Package version 3.1.

Redundancy Analysis (RDA): Canonical ordination analyses for relating the species composition of mollusc communities to their environment were carried out using CANOCO for Windows version 4.5 (Ter Braak & Šmilauer, 2002). The appropriate type of analysis (re-

TABLE 1. Physical and chemical parameters of water and their values (ranges) of the Mleczna River and mining subsidence reservoirs with flowing water compared to mining subsidence reservoirs with non-flowing water in the years 1993–2008.

Parameter	The Mleczna River (main overflow) No. 8	Reservoirs 1, 2, 3, 4 (flowing water)	Reservoirs 5, 6, 7 (non-flowing water)
Conductivity (μS/cm)	1800–3240	710–3020	290–1500
pH	7.0–7.9	7.0–8.3	7.1–8.4
Dissolved oxygen (mg O ₂ /l)	2.4–10.8	3.1–11.0	3.5–11.3
Nitrates (mg NO ₃ /l)	0.3–208.2	1.0–194.9	1.3–51.4
Phosphates (mg PO ₄ /l)	0.41–4.02	0.21–2.74	0.04–2.10
Total hardness (mg CaCO ₃ /l)	270–450	218–357	35–246
Iron (mg Fe/l)	0.19–1.50	0.09–1.50	0.01–1.39
Chlorides (mg Cl/l)	432–450	67–665	25–300
Alkalinity (mg CaCO ₃ /l)	260–365	140–390	85–225

dundancy analysis) was chosen to analyze the species data by DCA (Detrended Correspondence Analysis) and the length of the gradient. Preliminary DCA on the species data revealed that the gradient length was less than 3 SD (three standard deviation) indicating that the species exhibited linear responses to underlying environmental variation which justified the use of linear multivariate methods. Therefore, a linear direct ordination technique RDA with a forward selection was used to reduce the large set of environmental variables. The statistical significance of the relationship between the mollusc species and the physical and chemical parameters of water was evaluated using the Monte Carlo permutation test (499 permutations) (Ter Braak & Šmilauer, 2002).

Densities: Variations in densities of *P. antipodarum* at sampling sites in the mining subsidence reservoirs which receive water from a mine dewatering system and in the Mleczna River were recorded from June 2006 to June 2007.

The significance of differences in the density of *P. antipodarum* in the sampling sites were calculated by means of the Kruskal-Wallis one-way ANOVA test using the Statistica program version 8.0.

Shell Height and Number of Embryos: The significance of the differences in the shell height of *P. antipodarum* from particular sampling sites (four sampling sites) was calculated by means of the Kruskal-Wallis one-way ANOVA test. The relationship between the shell height

of *P. antipodarum* and the number of embryos in the brood pouch per female was calculated by means of the non-parametric test of the Spearman Rank Correlation Coefficient r_s . The data of the shell height and the number of embryos in the brood pouch of *P. antipodarum* did not reveal a normal distribution; therefore, non-parametric tests were applied. The statistical analysis was performed using the Statistica program version 8.0.

RESULTS

Water Physical and Chemical Parameters

The water from both the mining subsidence reservoirs with flowing water that receive water from a mine dewatering system and in the Mleczna River was high in conductivity, phosphates, chlorides and very high in nitrates compared to the reservoirs with non-flowing water (Table 1). The waters are soft and medium hard in reservoirs with non-flowing water (nos. 5, 6, 7), medium hard and significantly hard in reservoirs with flowing water and significantly hard or hard in the Mleczna River. The Kruskal-Wallis one-way ANOVA test revealed statistically significant differences in conductivity $H(2, N = 36) = 21.35$, $p = 0.0001$; alkalinity ($H = 21.04$, $p = 0.0001$); chlorides ($H = 20.04$, $p = 0.001$); total hardness ($H = 22.11$, $p = 0.0001$); iron ($H = 10.14$, $p = 0.01$) between the reservoirs with non-flowing and flowing water and the river. Nitrates ($H = 14.86$, $p = 0.001$) and phosphates ($H = 12.42$,

TABLE 2. Values of the dominance (D%) and the Shannon-Wiener (H') indices of the mollusc communities in mining subsidence reservoirs with flowing water.

Species	Years of survey												
	1993	1995	1996	1997	2000	2001	2002	2003	2004	2005	2006	2007	2008
<i>Viviparus contectus</i> (Millet, 1813)			6.6	0.4	0.8	2.9	2.4	3.4	5.5	0.6	0.8	0.3	0.4
<i>Bithynia tentaculata</i> (Linnaeus, 1758)					7.2	2.2	3.1	3.5	9.2	3.9	3.3	7.0	0.7
<i>Potamopyrgus antipodarum</i> (J. E. Gray, 1843)				83.0	51.8	66.4	38.4	40.5	43.5	81.4	85.1	89.9	96.5
<i>Acroloxus lacustris</i> (Linnaeus, 1758)	36.4	31.6	1.9	0.1	7.6	0.7		1.5	1.6	0.3	2.0	0.3	0.2
<i>Stagnicola palustris</i> (O. F. Müller, 1774)			3.8	0.4									
<i>Stagnicola corvus</i> (Gmelin, 1791)			1.9	0.5									
<i>Radix auricularia</i> (Linnaeus, 1758)					4.0		1.8	0.4	1.0	0.1	0.4	0.1	0.1
<i>Radix balthica</i> (Linnaeus, 1758)	24.5	24.7	22.6	1.6	4.8	1.9	4.3	3.2	12.6	1.0	0.6	0.2	0.2
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)	13.2	14.6	1.9	0.9	1.6	3.6	7.9	16.2	4.4	0.5	0.7	0.3	0.4
<i>Physella acuta</i> (Draparnaud, 1805)			9.4	2.4			1.8	0.3		0.5	0.8	0.3	0.3
<i>Aplexa hypnorum</i> (Linnaeus, 1758)									0.4	0.2	0.3	0.1	0.1
<i>Planorbarius corneus</i> (Linnaeus, 1758)	6.6	3.8	16.0	0.7	9.7	6.0	6.7	13.2	3.4	0.4	1.4	0.4	0.2
<i>Ferrissia wautieri</i> (Mirolli, 1960)							1.8	0.3					
<i>Anisus spirorbis</i> (Linnaeus, 1758)			3.8	2.3		0.5							
<i>Bathyomphalus contortus</i> (Linnaeus, 1758)				0.1					0.3		0.2	0.1	0.1
<i>Gyraulus albus</i> (O. F. Müller, 1774)	6.0	7.0	3.8	1.2	8.8	2.2	6.7	3.2	2.4	0.3	0.4	0.2	0.1
<i>Gyraulus crista</i> (Linnaeus, 1758)	1.3	6.3		0.2	1.6		3.7	1.8	7.8	4.2	0.5	0.2	0.1
<i>Hippeutis complanatus</i> (Linnaeus, 1758)			15.1	5.3	2.0	4.1	6.7	2.4	2.3	2.0	0.9	0.1	0.2
<i>Segmentina nitida</i> (O. F. Müller, 1774)	12.0	12.0	13.2	1.0		3.4	9.2	3.7	4.1	3.3	0.6	0.1	0.2
<i>Anodonta anatina</i> (Linnaeus, 1758)									0.6	0.2	0.3	0.1	0.04
<i>Sphaerium corneum</i> (Linnaeus, 1758)											0.8	0.2	0.1
<i>Musculium lacustre</i> (O. F. Müller, 1774)						4.8	3.1	2.1	0.4	0.2	0.5	0.1	0.1

(continues)

(continued)

Species	Years of survey												
	1993	1995	1996	1997	2000	2001	2002	2003	2004	2005	2006	2007	2008
<i>Pisidium casertanum</i> (Poli, 1791)						1.2	2.4	4.3	0.7	0.9	0.6	0.2	0.1
Σ of species	7	7	12	15	11	13	15	16	17	17	19	19	19
Σ of specimens	151	158	106	1,726	249	414	164	677	708	3,188	2,465	7,078	11,671
Value of the H'	2.36	2.49	3.22	1.18	2.44	2.03	3.22	2.89	2.88	1.80	1.16	0.69	0.41

$p = 0.002$) varied between the reservoirs with non-flowing water and the river.

Occurrence in Relation to Mollusc Communities and Values of Shannon-Wiener Index

Between 1993–2008, 23 species of molluscs were observed in the reservoirs with flowing water (Table 2), whereas only ten species were found in the Mleczna River (Table 3).

In 1997, *P. antipodarum* started to occur in the reservoirs with flowing water and in the Mleczna River, and it has become eudominant in the mollusc communities (Tables 2, 3). *Potamopyrgus antipodarum* is absent from the mining subsidence reservoirs with non-flowing waters.

The number of mollusc species increased from 1993–1996, reaching 15 in 1997 and collapsed to 11 in 2000. In the next few years, the number of species increased once again reaching 19 in 2006 (Table 2). Bivalve species: *Anodonta anatina*, *Sphaerium corneum*, *Musculium lacustre* and *Pisidium casertanum* were subprecedents in the mollusc communities from 2004 to the present in the mining subsidence reservoirs. In 1997–2008, the number of mollusc species increased from 3 to 10 in the Mleczna River (Table 3).

The Shannon-Wiener index values calculated for mollusc communities ranged from 0.41 to 3.22 in the reservoirs with flowing water and from 0.97 to 3.11 in the Mleczna River (Tables 2, 3).

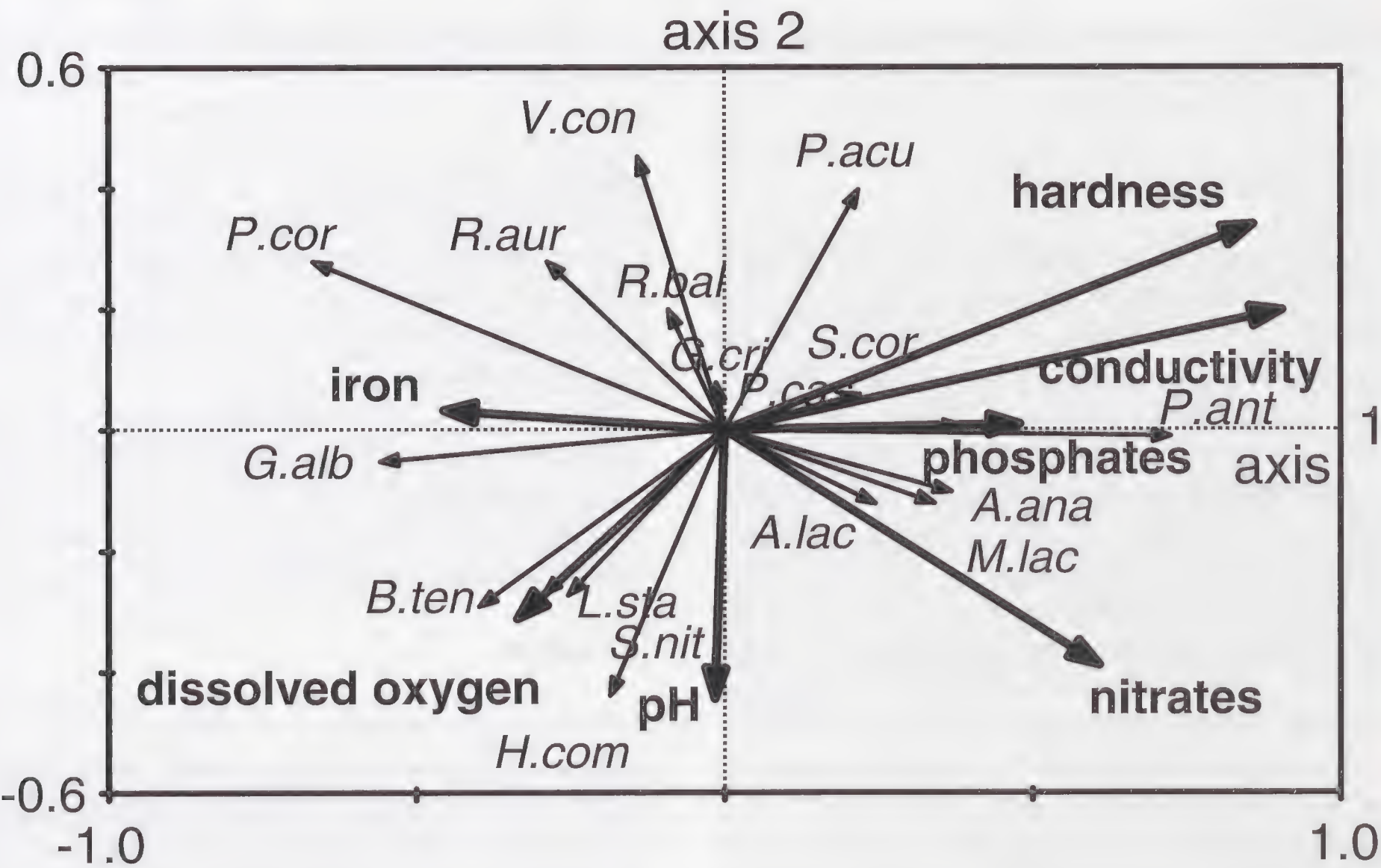


FIG. 2. Ordination diagram (biplot) based on a redundancy analysis (RDA) of the mollusca data including *P. antipodarum* and environmental variables. The long arrows representing some environmental variables emphasize their great impact on the community structure of Mollusca.

TABLE 3. Values of the dominance (D%) and the Shannon-Wiener (H') indices of the mollusc communities (Mleczna River).

Species	Years of survey												
	1993	1995	1996	1997	2000	2001	2002	2003	2004	2005	2006	2007	2008
<i>Viviparus contectus</i> (Millet, 1813)													3.5
<i>Bithynia tentaculata</i> (Linnaeus, 1758)						13.9	21.9	23.1	16.1	11.3	7.3	4.8	4.2
<i>Potamopyrgus antipodarum</i> (J. E. Gray, 1843)				47.2	39.3	41.7	25.0	26.92	29.0	22.6	13.8	48.7	11.9
<i>Radix auricularia</i> (Linnaeus, 1758)						8.3	15.6	15.4	19.4	13.2	11.0	5.9	2.8
<i>Radix balthica</i> (Linnaeus, 1758)	60	55.6	56.2	27.8	32.1	19.4	15.6	15.4	16.1	7.6	3.7	2.7	6.3
<i>Lymnaea stagnalis</i> (Linnaeus, 1758)	40	44.4	43.8	25.0	28.6	16.7	21.91	19.2	12.9	13.2	12.8	7.5	12.6
<i>Physella acuta</i> (Draparnaud, 1805)										20.8	44.0	24.6	50.3
<i>Gyraulus albus</i> (O. F. Müller, 1774)										3.8	2.8	2.7	4.9
<i>Sphaerium corneum</i> (Linnaeus, 1758)										3.8	2.8	1.6	2.1
<i>Musculium lacustre</i> (O. F. Müller, 1774)									6.4	3.8	1.8	1.6	1.4
Σ species	2	2	2	3	3	5	5	5	6	9	9	9	10
Σ specimens	15	18	16	36	28	36	32	26	31	53	109	187	143
Value of the H'	0.97	0.99	0.99	1.52	1.60	2.11	2.30	2.29	2.46	3.11	2.49	1.97	2.41

Redundancy Analysis (RDA)

Species and environmental variables based on the first two axes explain 38.95% of the variance in the species data and 89.05% of the variance in the fitted species data. Based on the redundancy analysis (RDA), the conductivity and the concentration of nitrates were the parameters most associated (statistically significant according to the forward selection results) with the distribution of mollusc species, including *P. antipodarum* (Fig. 2). The most important parameter contributing to RDA axis 1 was conductivity. Two different patterns of mollusc distribution were found: (1) *Potamopyrgus antipodarum* was the species situated close to the arrow indicating conductivity and *Gyraulus albus* was the species situated on the opposite side, (2) *Acroloxus lacustris* and the bivalves *Anodonta anatina* and *Musculium lacustre* were the groups of species situated close to the arrow indicating nitrates and *Plan-*

orbarius corneus was the species situated on the opposite side. Angles between the arrows of species and environmental variables in an ordination diagram from linear methods show a positive (acute angle) or a negative (straight angle) correlation.

The relation between the species and the environmental variables was statistically significant (test of significance of first canonical axis: $p = 0.016$, $F = 10.904$, test of significance of all canonical axes: $p = 0.012$, $F = 2.328$).

Densities

The mean density of *P. antipodarum* in the mining subsidence reservoirs with flowing water between June 2006 to June 2007 was $1,875.4 \pm 1,670.01$ individuals/m² ($\pm$ the standard deviation). The densities of *P. antipodarum* increased to 6,010 individuals/m² (Fig. 3) between June 2006 to August 2006. The densities showed a decreasing tendency from August

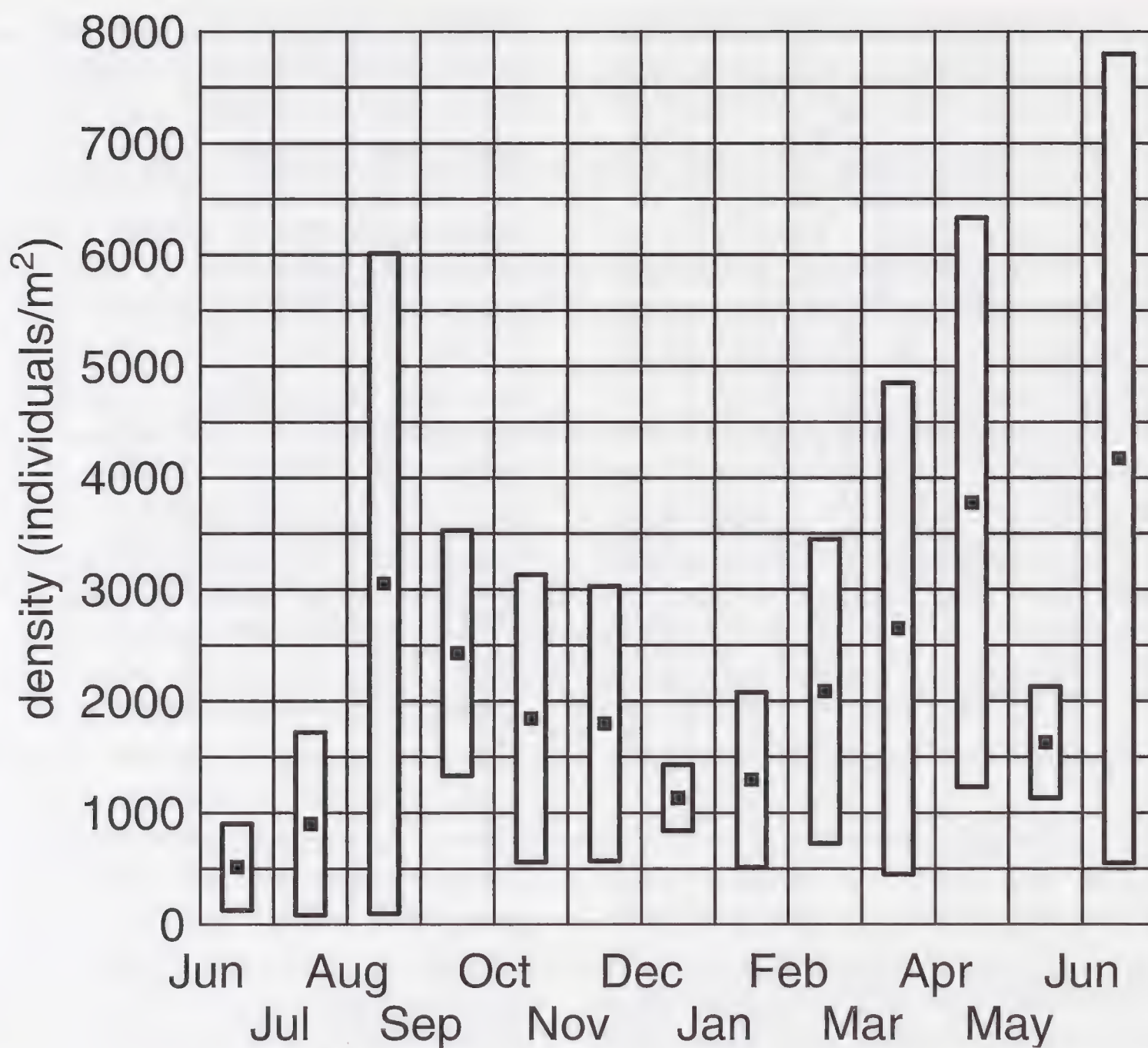


FIG. 3. Densities (mean and ranges) of *P. antipodarum* in the mining subsidence reservoirs with flowing water (individuals/m²), June 2006–June 2007; ■ = mean; □ = $\pm$ SD.

2006 to December 2006 (to 560 individuals/m² in December). From January 2007 the densities gradually increased again (Fig. 3). The lowest densities of 85 individuals/m² were recorded at sampling site 1c (July 2006), while the highest densities of 7,800 individuals/m² were recorded at sampling site 1b (June 2007). The Kruskal-Wallis one-way ANOVA test revealed a lack of statistically significant differences in *P. antipodarum* densities both at the sampling sites in particular months of the survey ($H(51, N = 52) = 51.0, p = 0.47$) and at sampling sites 1a, 1b, 1c, 3a in a particular month of the survey ($H(12, N = 13) = 12.0, p = 0.45$). The densities were low in the Mleczna River and ranged from 3 to 86 individuals/m².

Shell Height

The mean shell height of *P. antipodarum* varied depending on the sampling sites. The highest mean shell height of 4.42 ± 0.38 mm was recorded in October 2006 (sampling site 1b, submerged parts of leaves of *Typha latifolia*), while the lowest mean shell height of

3.31 ± 0.87 mm was recorded on submerged leaves of *Glyceria maxima* (site 1a) in January 2007. The maximum shell height was 6.96 mm (September, sampling site 1b). At sampling sites 1a and 1c the highest mean shell height was recorded in April 2007 and the lowest mean shell height in January. At sampling site 3a the highest mean shell height was recorded in June 2006. To conclude, low mean shell height was recorded twice in the year: in winter in January (sampling sites 1a and 1c) or February (sampling sites 1b and 3a) and in summer in July or August (sampling sites 1a, 1b and 1c). The Kruskal-Wallis one-way ANOVA test revealed statistically significant differences in the shell height of *P. antipodarum* among the sampling sites in each of the months of the survey ($p = 0.001$).

Number of Embryos in Brood Pouch

From June 2006 to June 2007 the mean number of embryos per female was 11.83 ± 10.23 ($\pm$ the standard deviation). The highest mean number per female was recorded in April 2007

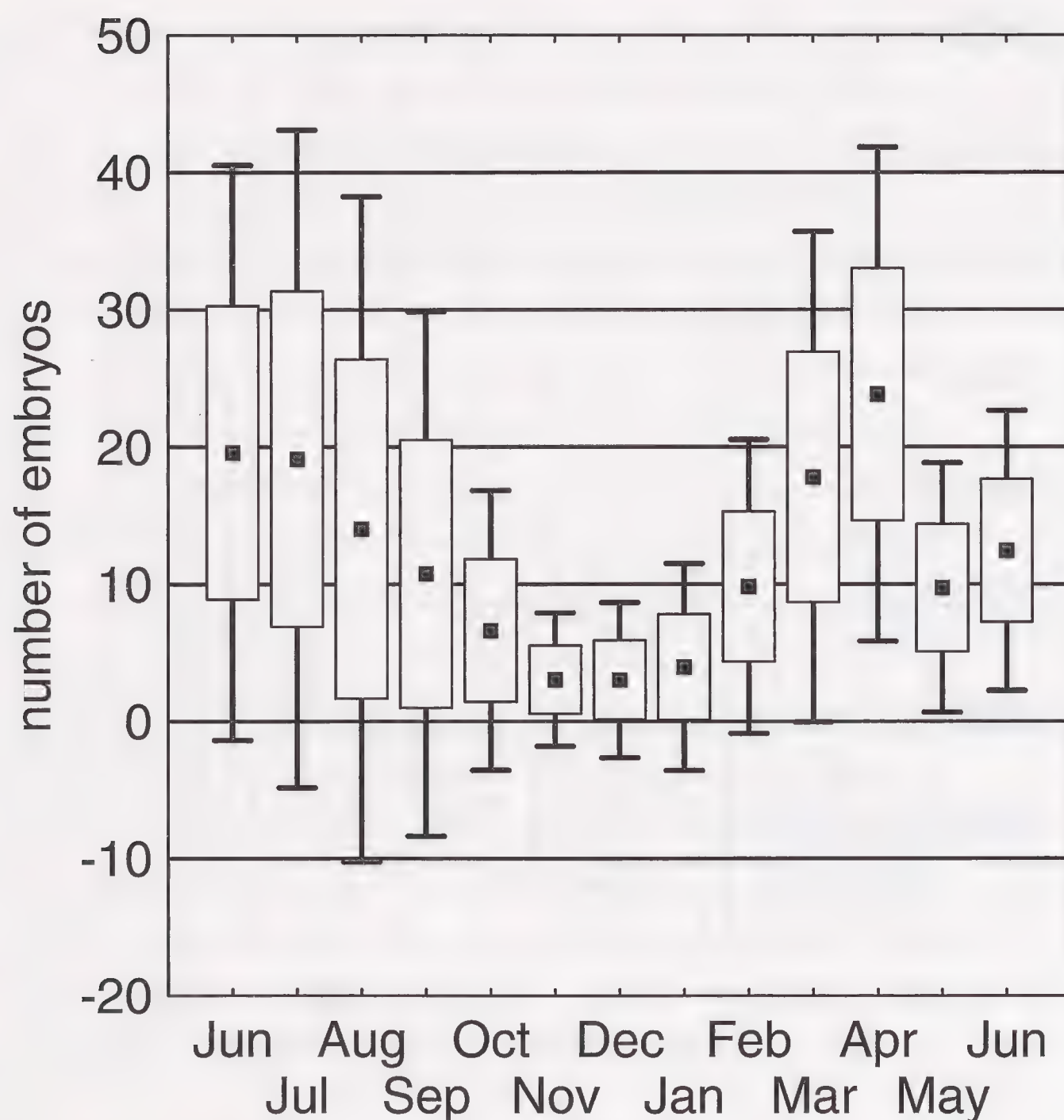


FIG. 4. The monthly mean number of embryos with SD in the brood pouch per female of *P. antipodarum*, June 2006–June 2007; ■ = mean; □ = $\pm$ SD; I $\pm$ 1.96 SD.

(23.83 ± 9.19); the lowest in December 2006 (3.02 ± 2.89) (Fig. 4). From December 2006 to April 2007, the number of embryos increased gradually, and subsequently decreased in May 2007. The maximum number of embryos amounted to 59 (April 2007). The embryos were recorded in the brood pouch in females with a shell height over 3.0 mm. Analysis of the Spearman Rank Correlation coefficient r_s showed a statistically significant correlation between shell height and the number of embryos per female in each of the months of the survey ($N = 120$, $p < 0.05$).

DISCUSSION

Invasion Stages within Mining Subsidence Reservoirs and the River

This long-term survey of the occurrence of *P. antipodarum* both in mining subsidence reservoirs and the Mleczna River in Upper Silesia disclosed novel findings about the invasion

process, from its initial dispersal to establishment and integration. According to Colautti & MacIsaac (2004) and Colautti et al. (2006), the invasion process is broken into stages from stage 0 (donor region) to stage V (widespread and dominant), with sequential filters that act to preclude transition between particular stages. The results of this study showed that *P. antipodarum* was not collected in 1996 and that within one year, a rapid increase in its density was observed (transformation from stage III: localized and numerically rare to stage IVb: localized but dominant). Probably, during the initial dispersal of *P. antipodarum* within the mining subsidence reservoirs or river, the population was too small to be detected (stage III). This survey confirmed a similar pattern of the occurrence of *P. antipodarum* at its initial dispersal and establishment stage in the water environments of Upper Silesia obtained by Strzelec & Krodziewska (1994).

Parthenogenesis and euryhalinity may explain the ability of *P. antipodarum* to increase in abundance and then lead to its successful

establishment (which could be expressed as its invasiveness characteristics or effectiveness of propagule pressure) within these reservoirs from 1996 to today. Considering the framework of Colautti & MacIsaac (2004), the population of *P. antipodarum* seems to be localized but dominant (stage IVb) within mining subsidence reservoirs with flowing water on a local scale. Reservoirs with non-flowing water compared to reservoirs with flowing water may be relatively invulnerable to invasions of *P. antipodarum*. It is possible that the movement of water both within subsidence reservoirs and in the Mleczna River is one of the dispersal vectors that enables *P. antipodarum* to spread. Other local dispersal vectors may also be important within mining subsidence reservoirs and the Mleczna River in addition to water movement, for example, fish and wildfowl, including migratory birds. These reservoirs are stocked with fish (Lewin & Smoliński, 2006), and many species of birds have been observed there. *Potamopyrgus antipodarum* may be ingested by them and may go through the digestive system unharmed to be excreted elsewhere or snails may be transported in mud on the feet or feathers of migratory birds or fishermen.

Invasive species must pass through at least three of the invasion stages before they are able to inflict ecological or economic harm, that is, transport, introduction, establishment, spread and impact (Lockwood et al., 2007). This survey did not show that *P. antipodarum* has had a negative influence on native mollusc species and at such a density is probably not able to inflict ecological or economic harm. However, a survey on the influence of *P. antipodarum* on other macroinvertebrates in these reservoirs was not carried out.

Occurrence in Relation to Mollusc Communities

These surveys found a few mollusc species that are rare for the Silesian Upland, southern Poland, that is, *Viviparus contectus*, *Physella acuta*, *Hippeutis complanatus*, and *Anodonta anatina*. Some of these are considered to be uncharacteristic species for this region, including *Bithynia tentaculata*, *Radix auricularia*, *Segmentina nitida*, *Musculium lacustre* and *Pisidium casertanum*. According to Strzelec (1993), *P. antipodarum* was a rare species in this region. This survey found the occurrence of *Bithynia tentaculata*, *Radix auricularia*, *Stagnicola palustris*, *Hippeutis complanatus* and *Ferrissia wautieri* for the first time in the mining subsidence reservoirs of Upper Silesia.

In 2000, 2004 and 2007 *Bithynia tentaculata* was dominant in the mollusc communities. *Ferrissia wautieri* was recorded in reservoirs with flowing water in the years 2002–2003. This species was first found in France in 1944 (Mirolli, 1960; Hubendick, 1972). *Ferrissia wautieri* is now considered to be a synonym of the North American *Ferrissia fragilis* (Tryon, 1863) (Walther et al., 2006; Walther, 2007). In 1997–2008, *P. antipodarum* was eudominant in the mollusc communities both in reservoirs with flowing water and in the Mleczna River. This result is consistent with the survey done by Strzelec & Krodzińska (1994) or Michalik-Kucharz et al. (2000) and indicates that there was an invasion of *P. antipodarum* into the water environments of Upper Silesia.

Influence of Physical and Chemical Parameters

The physical and chemical parameters of water play a certain role in the distribution of *P. antipodarum* within the study area. The Kruskal-Wallis one-way ANOVA test revealed statistically significant differences in conductivity, alkalinity, chlorides, total hardness, iron or biogenic elements among reservoirs with non-flowing and flowing water and the river. It seems that *P. antipodarum* prefers relatively higher values of these parameters. Thus, it inhabits reservoirs with flowing water that provide more chlorides from a mine dewatering system and the river that provides more biogenic elements compared to reservoirs with non-flowing water.

Potamopyrgus antipodarum tolerates a wide range of conductivity values from 41 to 3,593 $\mu\text{S}/\text{cm}$ (Costil et al., 2001; Gérard et al., 2003; Sousa et al., 2007b). In this survey, *P. antipodarum* was recorded in water with a relatively high conductivity (from 710 to 3,240 $\mu\text{S}/\text{cm}$). This result is consistent with Costil et al. (2001), who considered *P. antipodarum* characteristic for water with considerably higher conductivity values. Schreiber et al. (2003) also recorded its occurrence in rivers of high conductivity (up to 7,390 $\mu\text{S}/\text{cm}$). This survey showed that both conductivity and the concentration of nitrates were the parameters most associated (statistically significant) with the distribution of mollusc species, including *P. antipodarum*, in the reservoirs with flowing water. This result is opposite to those of Múrria et al. (2008), who showed negative correlations between the abundance of *P. antipodarum* and conductivity or ammonium concentrate in water, as well as that of Brzeziński & Kołodziejczyk (2001), who

obtained a negative correlation between its density and the total phosphorus. The results of this survey are consistent with Gérard & Dusart (2003), who showed that *P. antipodarum* density was highly statistically significantly correlated with the concentration of nitrates.

The influence of total hardness on mollusc distribution has been discussed by many authors. For example, Carlsson (2000) found *P. antipodarum* in very soft and soft waters (72.9–145.7 mg CaCO₃/l). In the British Isles, *P. antipodarum* inhabits both soft (Kerney, 1999) and hard water (Fish & Fish, 1989; Kerney, 1999). According to this survey, *P. antipodarum* occurred in medium hard and significantly hard waters in reservoirs as well in significantly hard or hard waters in the Mleczna River. These results confirmed the survey of Strzelec & Krodkiewska (1994), Strzelec & Serafiński (1996) and Strzelec (1999b) that *P. antipodarum* prefers hard or very hard waters.

Densities

The density of *P. antipodarum* out of its native range is differentiated from a few individuals/m² in Lake Erie (Levri et al., 2007); up to 49,260 individuals/m² in Lake Purumbete, Australia (Schreiber et al., 1998); 300,000 individuals/m² in the rivers and estuaries of the western part of the USA (Richards & Shinn, 2004) or in the Greater Yellowstone Ecosystem (Kerans et al., 2005) and more than 700,000 individuals/m² in the Upper Owens River watershed in California (Noda, 2007). Low densities of *P. antipodarum* (up to 86 individuals/m²) were recorded in the Mleczna River but there were relatively higher densities up to 7,800 individuals/m² in the mining subsidence reservoirs. This novel finding is in contrast to Strzelec (1993), who recorded densities of *P. antipodarum* from 33 to 999 individuals/m² in the mining subsidence reservoirs in Upper Silesia. High densities of *P. antipodarum* in streams seem to depend on a high primary productivity, constant water temperature and stable discharge (Richards & Shinn, 2004). For example, in geothermal spring streams (Yellowstone National Park) the densities of 500,000 individuals/m² may be explained by the warm and stable water temperature that has an elevated average temperature of 14.4°C in January, as well a bottom carpeted by filamentous algae and vascular plants (Hall et al., 2003). By contrast, long periods of drought, a highly variable water temperature and discharge regimes can reduce the density of *P. antipodarum* (Múrria

et al., 2008). Thus, relatively low densities and the fluctuations of *P. antipodarum* within the survey area throughout the year may be explained by the harsh winter conditions in Poland with an average snow-cover of 42–90 days and ice covering the surface of mining subsidence reservoirs for a few months, as well the management process during summer or autumn (removing macrophytes and regulating reservoirs' output).

This survey revealed a lack of statistically significant differences in *P. antipodarum* density both at the sampling sites in particular months of the survey and at the sampling sites with different types of substratum. These results are in contrast to Richards (2004), who recorded statistically significant differences in *P. antipodarum* densities and types of substratum.

Shell Height

This survey showed that the highest mean shell height (4.42 mm) and the maximum shell height (6.96 mm) of *P. antipodarum* occurred on submerged leaves of *Typha latifolia*, while the lowest mean shell height (3.31 mm) occurred on submerged leaves of *Glyceria maxima*. According to Brown (1997), gastropods prefer the wider leaf blades of macrophytes compared to narrow leaf blades because they provide more periphyton. *Potamopyrgus antipodarum* consumes periphyton, thus the wider leaf blades of *Typha latifolia* (sampling site 1b) probably provide more food (periphyton) than the narrow blades of *Glyceria maxima* (sampling site 1a) or a muddy-stony (sampling site 1c) as well muddy-sandy bottom (sampling site 3a). Thus, statistically significant differences in the shell height of *P. antipodarum* among the sampling sites may be explained by feeding (types of food). Recording the maximum shell height of 6.96 mm may also be explained by the detailed measurements of shell height throughout the year.

Within a native range, maximum shell height in populations of *P. antipodarum* ranges from 4 to 11.5 mm, whereas out of its native range it is almost half the size of specimens within a native range (Winterbourn, 1970). For example, the maximum shell height of *P. antipodarum* ranged from 4.0 to 6.0 mm in the British Isles (Wallace, 1979; Kerney, 1999). In Upper Silesia, the mean shell height ranged from 3.1 to 5.0 mm (rivers and anthropogenic reservoirs) with a maximum shell height of 5.0 mm (Strzelec & Serafiński, 1996; Strzelec, 1999a) compared to a maximum shell height of 5.8

mm in reservoirs with artificially heated water in central Poland (Berger & Dzieczkowski, 1977). This phenomenon, that is, the differences in the shell height of snails within a native range and out of their native range, may be explained by low temperatures as well the influence of poor food conditions (oligotrophic water, high altitudes versus eutrophic water and lowland water environments) on small shell height. Large shell height is a result of higher temperatures, which may permit an increase in the rate and amount of growth (Winterbourn, 1970). For example, the mean annual air temperature in New Zealand ranges from 10°C to 16°C, whereas in Poland it only ranges from 7°C to 9°C. In addition, the larger shell height is more a result of an increase in the number of whorls than in an increase in the size of whorls (Winterbourn, 1970).

Number of Embryos in a Brood Pouch

In Australia, Schreiber et al. (1998) found a maximum number of embryos per female in a brood pouch of 42: the lowest number of embryos was recorded in autumn and in winter, while the highest number was recorded in spring. The results of this survey showed a similar tendency, but the maximum number of embryos was 59 (April). The maximum number of 59 falls between the maximum values recorded by Strzelec & Serafiński (1996), that is, 56 embryos (river) and 66 (sand-pit reservoir) in Upper Silesia. The highest mean number of embryos per female was recorded in April (23.83 embryos), but this is only half the number obtained by Strzelec & Serafiński (1996). In the USA, the number of embryos in a brood pouch found by Cada (2004) ranged from 1 to 83. This survey showed the mean number of embryos of 11.83 ± 10.23 , almost three times lower than that recorded in the USA (Cada, 2004).

Within a native range of *P. antipodarum*, the maximum number of embryos per female amounts to over 100. The number of embryos in the brood pouch is probably a function of the size of the shells (and therefore the brood pouch) (Winterbourn, 1970). In Poland, relatively low temperatures and low food availability during winter may explain the reduction in the growth of snails and therefore the number of embryos in the brood pouch.

A novel finding of this survey showed that *P. antipodarum* produces embryos throughout the year with the lowest mean embryo number recorded in winter (December).

Interaction with Native Freshwater Mollusca

Strzelec (2005) stated that in the anthropogenic reservoirs of Upper Silesia, *P. antipodarum* with a density above 100 individuals/m² may have influenced a decrease in the number of native snail species. In those reservoirs, the increase in the abundance of *P. antipodarum* influenced a decrease in the abundance of *Radix balthica*, *Planorbis planorbis*, *Acroloxus lacustris*, *Segmentina nitida*, *Bathyomphalus contortus* and *Gyraulus albus*. Piechocki & Kaleta (2001) also claimed that a mass occurrence of *P. antipodarum*, with a density of 30,000 individuals/m², may restrict the number of other mollusc species and their abundance. However, their results concerned natural lakes and require further research. This survey does not suggest that *P. antipodarum* with a maximum density of 7,800 individuals/m² has a negative influence on native mollusc species. This result is opposite to Strzelec (2005) because *P. antipodarum* co-occurred with *Acroloxus lacustris*, *Bithynia tentaculata*, *Radix balthica* or *Gyraulus albus* not only in the mining subsidence reservoirs but also in the Mleczna River. A gradual increase in other mollusc species numbers has been observed in these aquatic environments. This result is consistent with Schreiber et al. (2003), who showed a lack of a significant relationship between the presence of *P. antipodarum* and native freshwater snails, and in contrast to that of Ponder (1988) who obtained a negative relationship between the New Zealand mud snail and native snail species. However, it was shown that *P. antipodarum* significantly limited the growth of native snails, whereas the native snails facilitated the growth of the *P. antipodarum* (Riley et al., 2008).

The results of this survey showed the same pattern of densities as stated by Múrria et al. (2008): higher population densities in summer, no effect or a small effect on native Mollusca and stream structure because of smaller *P. antipodarum* densities compared with the western United States survey, where the New Zealand mud snail has a strong influence on stream function including native Mollusca species or other macroinvertebrates (Hall et al., 2003; Kerans et al., 2005; Hall et al., 2006).

CONCLUSIONS

A long-term survey on the occurrence of *P. antipodarum* both in mining subsidence res-

ervoirs and the river in Upper Silesia, which had not been carried out to date permitted an analysis of its initial dispersal, establishment and integration.

Beginning with the initial dispersal in 1997, *P. antipodarum* occurred only in the reservoirs with flowing water and in the Mleczna River. Currently, *P. antipodarum* has been eudominant in the mollusc communities (stage IVb: localized but dominant).

Potamopyrgus antipodarum has established itself in aquatic environments with a wide range of values of many physical and chemical parameters, including conductivity, chlorides, phosphates or the concentration of nitrates in water. The fluctuation in the occurrence of molluscs including *P. antipodarum* may result from the physical and chemical parameters of the water of the mining subsidence reservoirs, which receive water from a mine dewatering system, the management process, and reclamation of the reservoirs. Based on a redundancy analysis (RDA), the conductivity and the concentration of nitrates were the parameters most associated with the distribution of mollusc species including *P. antipodarum*.

The results of this survey revealed statistically significant differences in the shell height of *P. antipodarum* depending on the type of substratum in each of the months of the survey. The maximum shell height amounted to 6.96 mm and was bigger than the maximum shell height of specimens recorded in Poland to date (5.5–5.8 mm).

In the mining subsidence reservoirs in Czułów, *P. antipodarum* is able to reproduce throughout the year. The maximum number of embryos in a brood pouch per female was 59, which is lower than the maximum number of embryos of specimens which occur both in New Zealand (over 100 embryos) and other aquatic environments in Poland (63–66 embryos).

The results of this survey do not suggest that the integration of *P. antipodarum* has negatively influenced the occurrence of other mollusc species either in the mining subsidence reservoirs or in the Mleczna River, probably due to their lower densities compared with the western United States survey, where *P. antipodarum* has a strong influence on aquatic ecosystems and restricts the occurrence of the native macroinvertebrates. *Potamopyrgus antipodarum* has established itself in the mining subsidence reservoirs and integrated itself into the local molluscan species without any major negative effects (e.g. extirpations) on the communities being invaded. However, the ability to

predict the effect of biotic invasions into aquatic ecosystems is still limited. The survey on the establishment and integration of *P. antipodarum* both in the mining subsidence reservoirs and in the Mleczna River should continue.

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EFFECT OF FOOD AVAILABILITY ON MORPHOMETRIC AND
SOMATIC INDICES OF THE APPLE SNAIL *POMACEA CANALICULATA*
(CAENOGASTROPODA, AMPULLARIIDAE)

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ABSTRACT

Pomacea canaliculata is a freshwater snail native to South America that together with some congeners has invaded natural wetlands and paddy fields in several continents, especially in Southern Asia. The high variability in shape, color and thickness of *Pomacea* shells and the sexual dimorphism in many traits blurs the species limits and hampers taxonomic identification. Ecological characterization of habitat productivity based on shells was previously proposed for *P. canaliculata* but was never methodically explored. Using full siblings of *P. canaliculata*, we studied the effects of different chronic levels of food availability (from 100% to 20% of daily ingestion rate) on shell shape, somatic indices and sexual dimorphism at maturity. The eight specific morphometric and somatic indices investigated showed different combinations of the effects of food availability and sex: changes related to food availability but independent of sex (relative aperture width), sexual dimorphism independent of food availability (shell globosity and relative aperture expansion), and changes related to food availability and sex, without a noticeable interaction (organic density); a significant interaction that increases the intersexual differences when food availability increases was detected in some indices (relative operculum weight, overall shell density and relative shell investment). The organic density can be used as a condition index to indicate the actual trophic availability in the field, although it should be estimated separately for males and females. The relative aperture width and the overall shell density can be used as paleo-environmental indicators of productivity, as they can be measured on empty shells. The effect of water alkalinity should be taken into account should the latter be used.

Key words: freshwater snail, shape, shell, invasive, organic density.

INTRODUCTION

Apple snails have invaded freshwater habitats of Southeast Asia, North America and the Pacific islands (Cowie, 2002; Hayes et al., 2008; Rawlings et al., 2007), causing economic losses in aquatic crops of several billion dollars (Joshi & Sebastian, 2006); one of the major pests in this group seems to be *Pomacea canaliculata* (Lamarck, 1822) (Cowie et al., 2006; Hayes et al., 2008). The shell of *P. canaliculata* is highly variable in shape, thickness, color and banding pattern among populations and also between sexes (Cazzaniga, 1990; Estebenet, 1998; Estebenet & Martín, 2003; Estebenet et al., 2006), which has contributed to taxonomic confusion in this genus (Cazzaniga, 2002; Cowie et al., 2006).

The inter-population variation in shell shape in *P. canaliculata* has both genetic and environmental causes (Estebenet & Martín, 2003; Estebenet et al., 2006), but the response to specific environmental factors has not been experimentally investigated. Food availability is an environmental factor that shows a strong effect on several life history traits of this species, including growth rates and age and size at maturity (Estoy et al., 2002a, b; Tamburi & Martín, 2009a, b). In other snails (Urduy et al., 2010), theoretical models and empirical evidence have suggested that growth rates can interact with allometric growth patterns to produce different shell shapes.

Adults of different populations of *P. canaliculata* have significant sexual dimorphism in size and shell shape (Cazzaniga, 1990; Estebenet,

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1998; Estebenet et al., 2006), with a variable degree of expression (Estebenet et al., 2006). The dimorphism in aperture shape in this species has been attributed to the development of the penis sheath (Cazzaniga, 1990; Estebenet, 1998), a voluminous ancillary copulatory organ on the mantle edge of males (Gamarra-Luques et al., 2006). *Pomacea canaliculata* males always mature at the same age under a gradient of food availability, but size at maturity decreases with deprivation (Estoy et al., 2002a; Tamburi & Martín, 2009a). Therefore, it is likely that food availability could explain a significant fraction of the variation in sexual dimorphism in shell shape through the effect on male size at maturity.

Estebenet & Martín (2003) suggested that *P. canaliculata* individuals growing at high rates develop thinner shells than those growing slower. The large investment in CaCO_3 to build egg shells (Cazzaniga, 1990) and to provide the embryos with storage for initial shell growth (Turner & McCabe, 1990) leads to the prediction that females should develop thinner shells than males, as previously observed by Cazzaniga (1990). Intersexual differences in shell thickness should increase with food availability due to the higher reproductive output in better fed females (Tamburi & Martín, 2011).

On account of the effects of *P. canaliculata* and congeners on natural wetlands (Carlsson et al., 2004; Cowie et al., 2006; Rawlings et al., 2007), it is important to know the actual trophic availability these apple snails have in the field, since it would be helpful to predict population growth rates on the basis of age at maturity and fecundity (Estoy et al., 2002b; Tamburi & Martín, 2009, 2011). However, it would be misleading to estimate such availability from aquatic vegetation biomass or cover, due to the different palatabilities of aquatic plants (e.g., Boland et al., 2008; Estebenet, 1995; Morrison & Hay, 2010; Qiu & Kwong, 2009) and the diverse feeding mechanisms (Cazzaniga & Estebenet, 1984) of this species. Consequently, an index of nutritional condition that reflects the food availability actually experienced by apple snails would allow a better prediction of their future impact on the aquatic vegetation of recently invaded water bodies. On the other hand, morphometric indices or marks that are measurable or recordable in apple snails when the shell is the only structure available for study have been considered of potential interest for paleo-environmental reconstruction (Martín & De Francesco, 2006; Van Damme & Pickford, 1995).

The main aim of this study was to test experimentally if food availability has an effect on shell shape and relative weight of *P. canaliculata* at maturity, through the response of several morphometric and somatic indices to different levels of food availability in each sex.

MATERIALS AND METHODS

The experimental design has been described in previous studies, in which the effect of food availability on age and size at maturity (Tamburi & Martín, 2009a), growth rates (Tamburi & Martín, 2009b) and reproductive output (Tamburi & Martín, 2011) of *Pomacea canaliculata* was studied. In order to minimize genetic variation, and to focus on environmental effects, snails used in the study were full siblings that came from a single egg mass laid by a laboratory reared female that only had contact with one male, both from the Curamalal Grande stream (36°57'36"S, 62°9'31"W, Buenos Aires Province, Argentina). All the hatchlings from the egg mass were reared during three weeks in a 20 liter collective aquarium. After this initial period, 72 of these snails were randomly selected (mean shell length $\pm$ SE: 4.96 mm $\pm$ 0.056, $n = 25$), and reared thereafter in isolation in 3 liter aquaria. Aquaria were filled with tap water saturated with CaCO_3 and placed in a rearing chamber that maintained homogeneous conditions of $25 \pm 3^\circ\text{C}$ and a 14 h : 10 h (light:dark) photoperiod; the snails were fed only with fresh lettuce.

The snails were randomly assigned to seven different levels of relative food availability (FA%). An empirical equation with shell length (SL) as the predictive variable (i.e., $\text{FA100\%} = 0.0033 \cdot \text{SL}^{1.9322}$; Tamburi & Martín, 2009b) was used to predict the daily amount of drained fresh lettuce ingested by a snail of a given size (FA100%, g) and the other levels of relative food availability (FA%: 87, 73, 60, 47, 33 and 20%) were calculated accordingly. The weekly ration was calculated according to the mean shell length of all snails assigned to each FA% level in a given week, and it was provided in two half-doses on Tuesdays and Fridays. Once a week the water was changed, aquaria were cleaned, and their position within the rearing chamber was changed randomly; the total shell length of each snail (TL, Fig. 1) was measured using a micrometric lens up to the sixth week or with a caliper thereafter. On each occasion, snails were inspected to detect their sex as

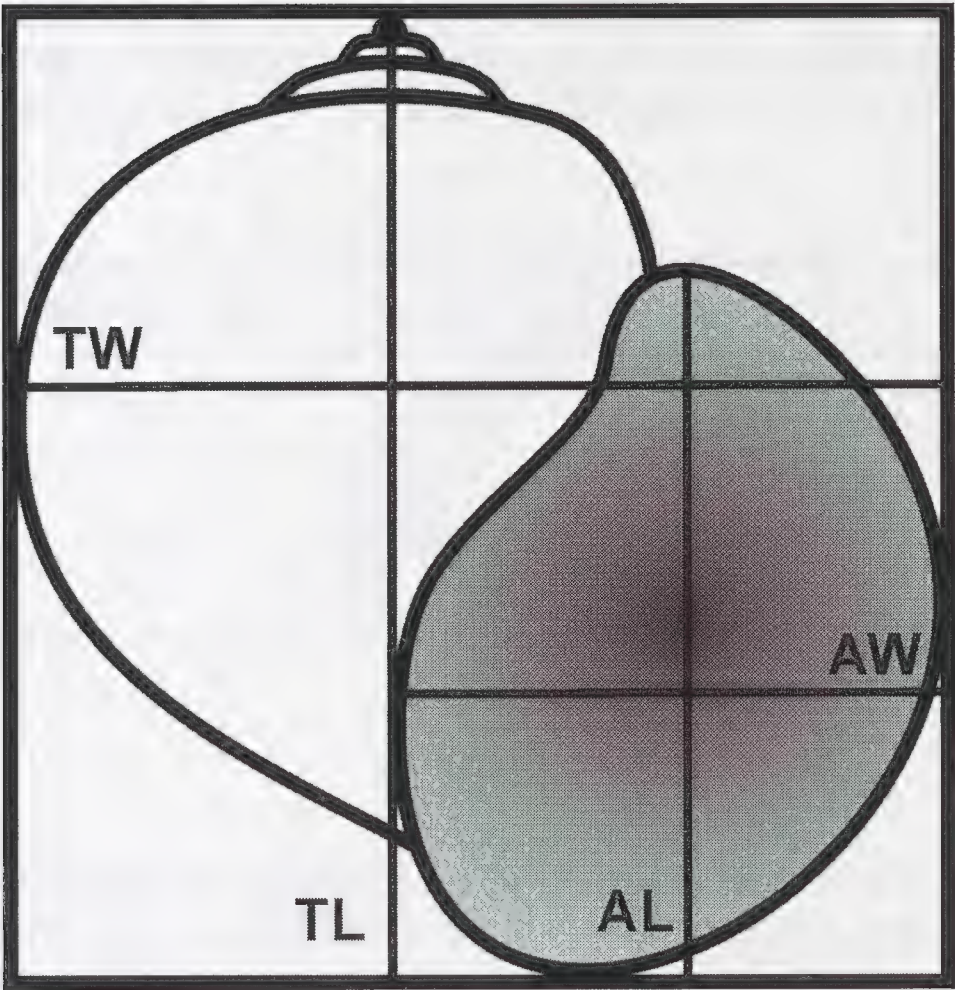


FIG. 1. Morphological measurements on the shells of *P. canaliculata*: total length (TL), aperture length (AL), total width (TW) and aperture width (AW).

early as possible through observation of the testicle through the shell or by the humping of the operculum in males. The number of male snails was equal (five) for each food availability level but, due to the random assignment of hatchlings to treatments, the number of females fluctuated, being 2, 3, 4, 4, 6, 6 and 3 from 100% to 20% FA respectively.

An external set of mature consort snails (three months older and fed with lettuce *ad libitum*) was used to detect the maturity of the experimental snails (i.e., copulatory behavior and egg laying). The randomly assigned consort snail was put into the experimental snail’s aquarium for only 24 h a week (after the water

change), to minimize trophic interferences and the experimental snail was fed after the consort was retrieved.

Four weeks after the experimental snails reached maturity (first egg mass laid by experimental or consort females) they were sacrificed by immersion in water at 80°C and stored at -16°C for later dissection. The body was extracted from the shell and dried at 80°C for 48 h, weighed and then calcinated at 600°C for 4 h and the ash weight was obtained using an analytical scale (± 0.0001 g). The organic weight was calculated by the difference between the dry and ash weight. The shell and the operculum were weighed after 48 h at 80°C; the internal volume (V, mm³) of the shell was estimated by the difference between the weight of the empty shell and its weight after filling it carefully with distilled water (assuming a density of 1 g/cm³); this procedure of volume estimation was repeated twice and the results averaged. Digital images of the shell in apertural view were obtained and four linear dimensions of the shell (Fig. 1; Estebenet & Martín, 2003) were measured on the digital photographs with SigmascanPro® software.

Four indices of shell morphology were generated for studying the food availability and sexually related changes. The shell globosity (SHG) was defined as the total width (TW) relative to the total length (TL); the relative aperture expansion (RAE) as the aperture width (AW) relative to the total width (TW); the relative aperture width (RAW) as the aperture width (AW) relative to total length (TL) and the relative aperture shape (RAS) as the aperture width (AW) relative to the aperture length (AL).

Four somatic indices based on different combinations of volume, weight and linear measurements of the snails were calculated:

TABLE 1. Tests (p values) for morphometric and somatic indices at maturity in males and females of *P. canaliculata* reared under different food availabilities (x: Cuzick test was not performed if the effect of food availability was not significant). Shell globosity (SHG), relative aperture expansion (RAE), relative opercular weight (ROW), overall shell density (OSD), organic density (OD).

	Variable	SHG	RAE	ROW	OSD	OD
Kruskal-Wallis Test	Males	0.064	0.239	< 0.001	< 0.005	0.035
	Females	0.768	0.273	0.614	< 0.005	0.025
Cuzick Test	Males	x	x	< 0.001	< 0.001	< 0.001
	Females	x	x	x	< 0.001	< 0.001
t-Test between sexes		< 0.001	< 0.001	< 0.001	0.313	< 0.001

TABLE 2. Parametric tests (p values of two and one way ANOVAs) for morphometric and somatic indices at maturity in *P. canaliculata* for males and females reared under different food availabilities (FA%) (x: not performed). Relative aperture width (RAW), relative aperture shape (RAS), relative shell investment (RSI).

Variable	RAW	RAS	RSI	RSI (Males)	RSI (Females)
Transformation	None	None	ArcSin	ArcSin	Ln
Sex	0.851	0.095	0.069	x	x
FA%	< 0.005	0.112	< 0.001	0.451	< 0.01
Sex x FA%	0.657	0.259	< 0.005	x	x

the relative shell investment (RSI) was defined as the shell dry weight relative to the body ash weight, the relative opercular weight (ROW) as the operculum dry weight relative to the aperture product (AL x AW); the overall shell density (OSD) was defined as the shell dry weight relative to the internal volume of the shell (V) and the organic density (OD) was defined as the body organic weight relative to the internal volume of the shell (V).

The statistical analyses were performed through two-way ANOVAs when the homogeneity of variances was not rejected, or when it was attained after transformation (as suggested by the Box & Cox method). In the case that this assumption was not fulfilled, one-way non-parametric Kruskal-Wallis tests were carried out for each sex, since two-way Kruskal-Wallis tests could not be performed due to unequal cell sizes (Zar, 1984). In these instances, t-tests for unequal variances for the average of each sex and Cuzick’s test for trends (Cuzick, 1985) were calculated to obtain more detailed information on the interaction between sex and food availability.

Voucher specimens have been deposited in Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN, Buenos Aires, Argentina). The specimens were juvenile (full-siblings of experimental snails) and adult snails (collected at the Curamalal Grande stream) registered under the collection numbers MACN-In 39436 and MACN-In 39437, respectively.

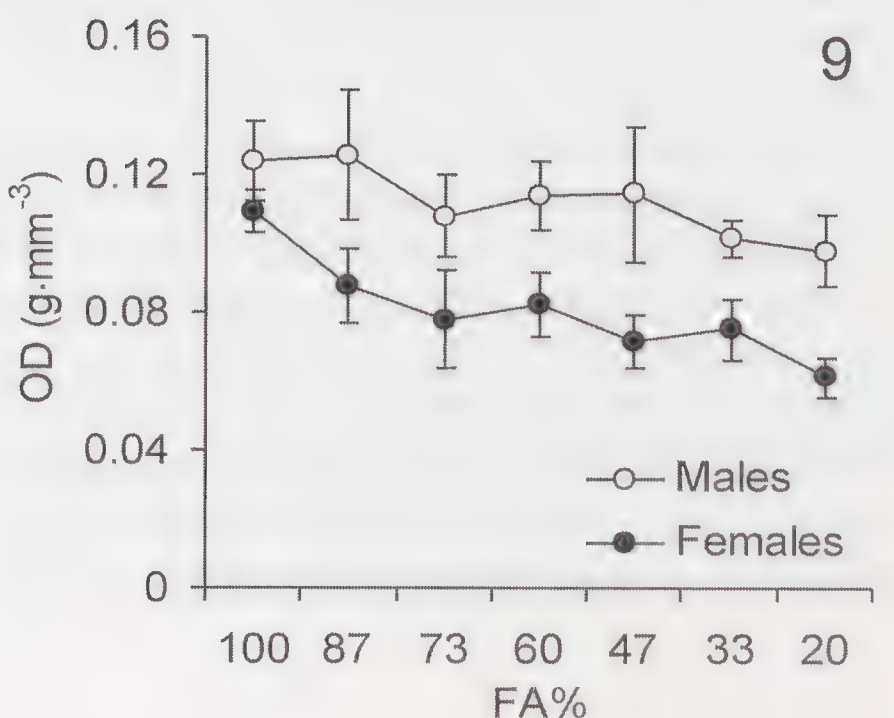
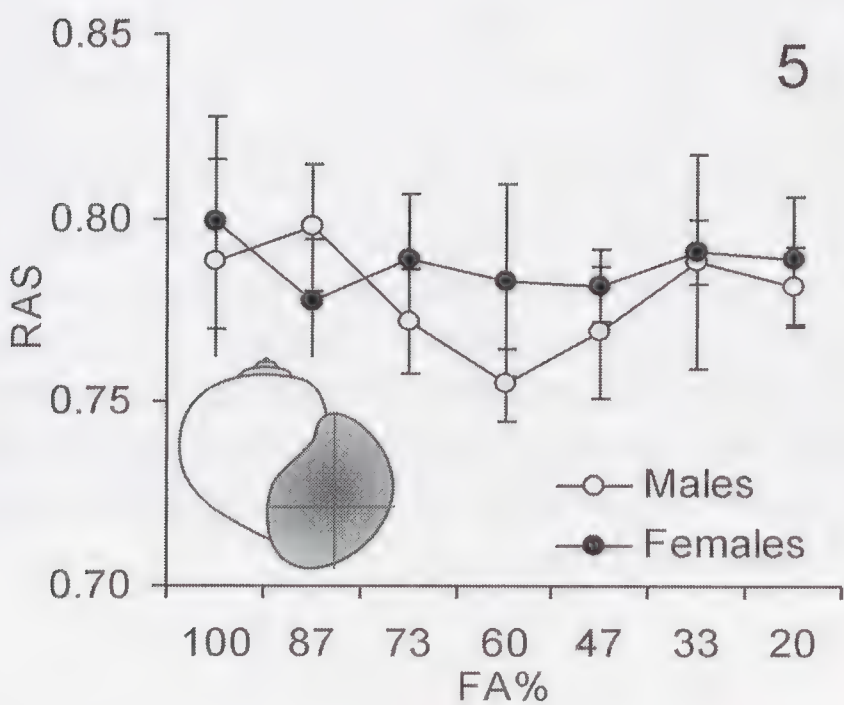
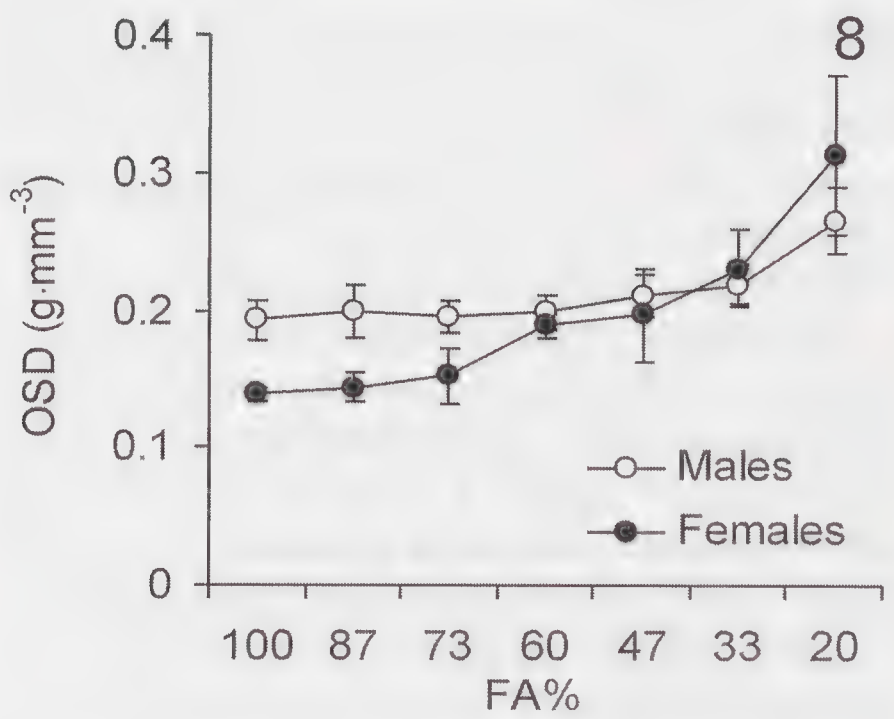
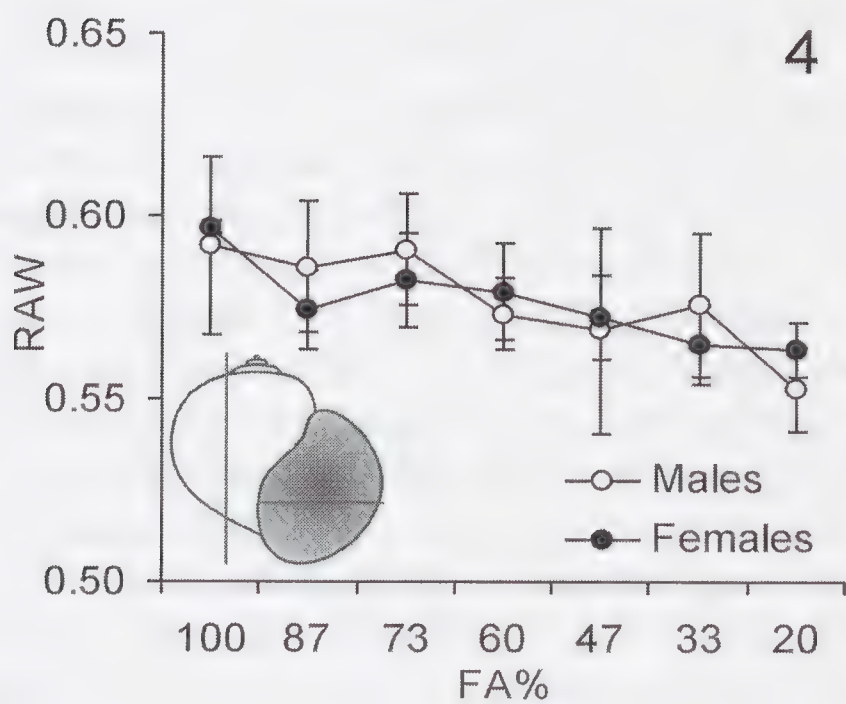
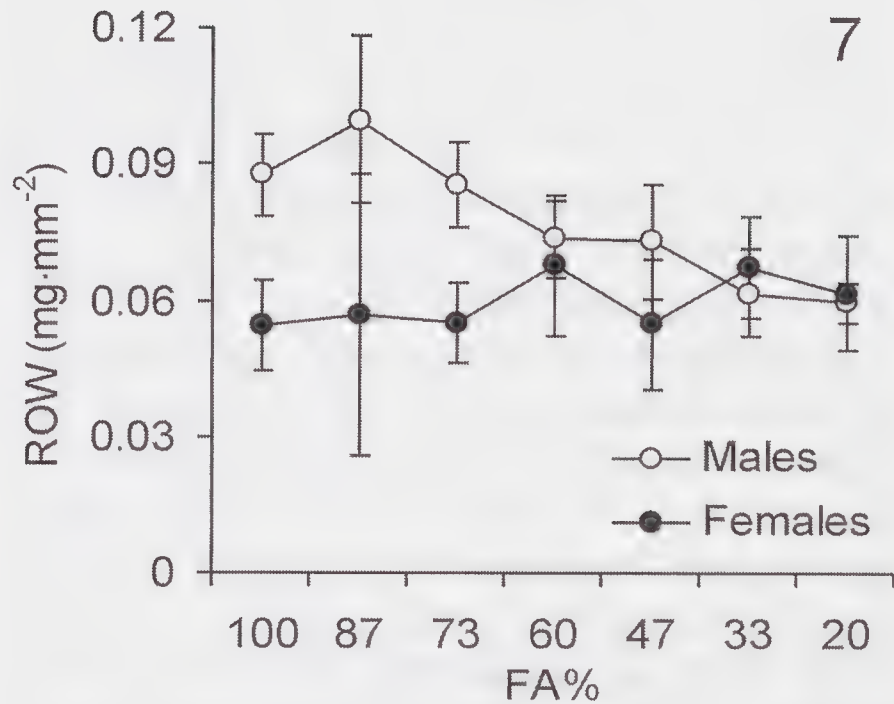
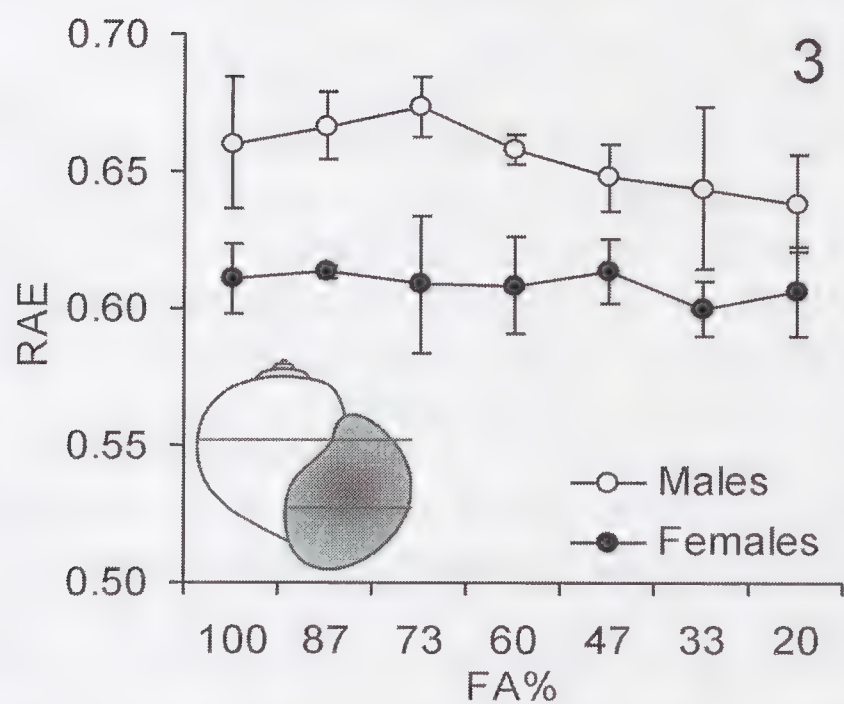
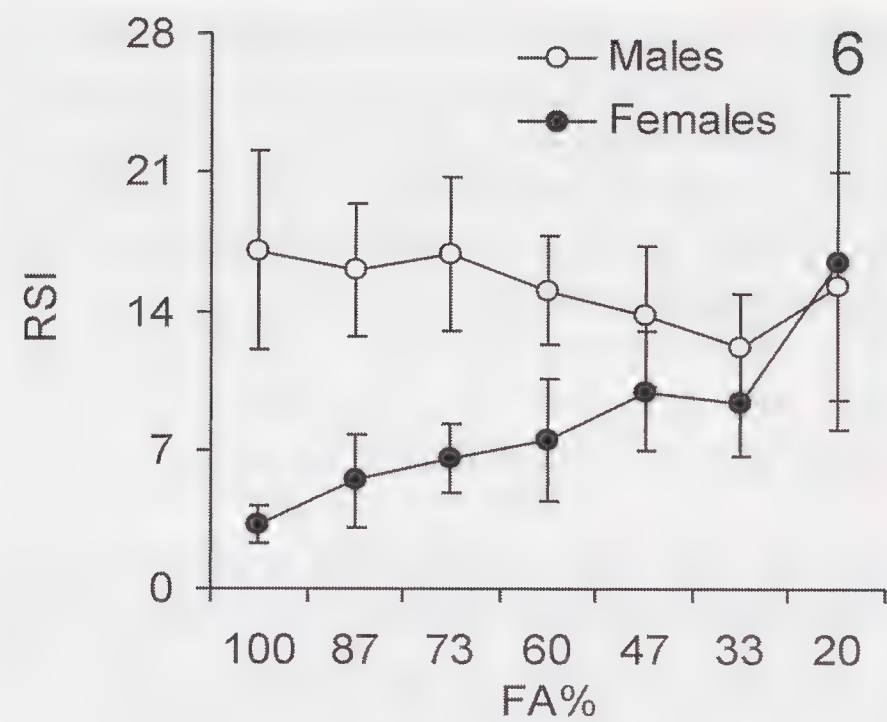
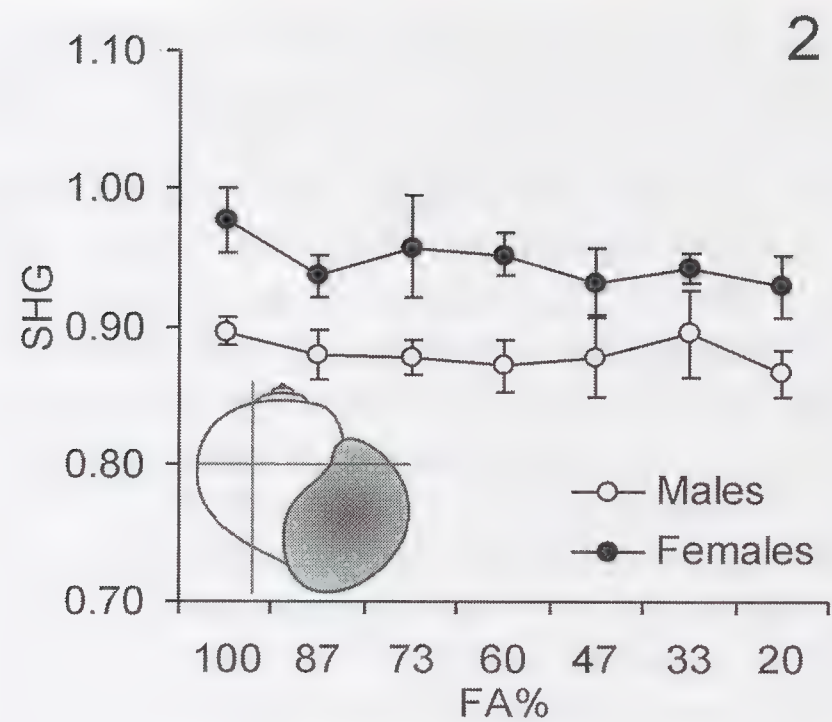
RESULTS

Pomacea canaliculata females showed a more globose shell (higher SHG; Fig. 2) and a relatively smaller aperture expansion (lower RAE) than males, irrespective of food availability (Fig. 3; Table 1). The relative aperture width (RAW) did not show any sexual dimorphism but increased with food availability in both sexes (Fig. 4; Table 2), while the relative aperture shape (RAS) did not show sexual dimorphism or any effect related to food availability (Fig. 5; Table 2).

The relative shell investment (RSI) was independent of food availability in males and on the whole was higher than in females, in which this somatic index decreased with the increase in food availability (Fig. 6; Table 2). Conversely, in females the relative opercular weight (ROW) was independent of food availability and smaller on average than in males, in which it increased with food availability (Fig. 7; Table 1); the differences between sexes increased with food availability. The overall shell density (OSD) increased with food availability without significant sexual dimorphism in the grand mean of all treatments (Fig. 8; Table 1). Nevertheless, some degree of interaction between sex and food availability was apparent, so t-tests for unequal variances for comparing sexes at each food availability level were performed; the three higher food availability levels (100, 87, 73% FA) showed significant differences (global error corrected by Dunn-

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FIGS. 2–9. Morphometric and somatic indices (mean ± SD) for *P. canaliculata* males and females at maturity depending on the food availability (FA%). FIG. 2: Shell globosity (SHG), FIG. 3: Relative apertural expansion (RAE), FIG. 4: Relative apertural width (RAW), FIG. 5: Relative apertural shape (RAS), FIG. 6: Relative shell investment (RSI), FIG. 7: Relative opercular weight (ROW), FIG. 8: Overall shell density (OSD), FIG. 9: Organic density (OD).



Sidak $p = 0.016$) while the remaining four (60, 47, 33, 20% FA) were not significantly different ($p > 0.05$ in each comparison). The organic density (OD) increased with food availability in both sexes, on average being higher in males than in females (Fig. 9; Table 1).

DISCUSSION

Our study showed different combinations of the effect of food availability and sex (single or double, additive or non additive) on eight morphometric and somatic indices for recently matured *Pomacea canaliculata* snails. Among morphometric indices, only the relative aperture width (RAW) was affected significantly by food availability. Several studies have shown a relationship between shell shape and growth rates in different snails (e.g., Chapman, 1997; Kemp & Bertness, 1984; Vermeij, 1980), which may be related to the interplay with allometric growth patterns (Urdu et al., 2010). Growth rates for the experimental snails studied here were strongly affected by food availability: the time required to attain a shell length of 22 mm increased more than three fold with a decrease of 80% in trophic availability (Tamburi & Martín, 2009b). Estebenet (1998) investigated the allometric growth in several dimensions of the shell of *P. canaliculata* and the reported deviations from isometry were mostly small (1.5% to 7%), probably accounting for the slight overall effect of growth rates on shell shape.

The relative opercular weight (ROW) showed no significant effect of food availability in females, probably indicating that the operculum area is proportional to that of the shell aperture. In the case of males, this index increases with food availability, without changes in aperture shape (RAS), probably reflecting an accelerated humping (Estebenet et al., 2006) or thickening of the operculum in males at high growth rates. The values of relative opercular weight for males in the two worst fed treatments were similar to those of females.

The pattern of overall shell density (OSD) relative to food availability indicates that the snails growing at higher rates have thinner shells. This inverse relationship between shell thickness and growth rate in *P. canaliculata* has been attributed to faster progress of the mantle edge (the zone of more intense deposition of CaCO_3) as the growth rate increases (Estebenet & Martín, 2003) and is apparently common among

snails (Urdu et al., 2010). In *P. canaliculata* females, the albumen gland complex stores large amounts of CaCO_3 (Catalán et al., 2006) and this constitutes most of the female's body ash content (Estebenet & Martín, 2003). The overall shell density only showed differences between sexes at the highest food availabilities, suggesting that the growth of the albumen gland complex is only detrimental to shell growth in these treatments, probably due to limitation in the calcium absorption rate (even in CaCO_3 saturated water). The higher levels of shell CaCO_3 relative to those of the soft parts (RSI) of females at low food availabilities are probably related to the smaller albumen glands in females deprived of food (Estoy et al., 2002b).

Youens & Burks (2008) stressed the need for standardized and reliable measurements of size and weight in order to obtain comparable estimates of individual and population biomass in *Pomacea*. In addition, the effects of environmental factors need to be considered in order to reduce, or to understand, the variability in these parameters. For instance, Estebenet & Martín (2003) suggested that the organic weight of the soft parts (adjusted by shell length) of *P. canaliculata* females increases with the productivity of the water bodies in which they live. Similarly, our results indicate that OD (organic weight relative to shell volume) of both females and males increases with food availability. These results suggest that the growth of shell volume is less affected by food availability than the overall growth of soft parts, probably combining lower amounts of stored reserves and muscle or an atrophied digestive gland. The smaller values for females are probably due to the large portion of their internal volume that is occupied by the albumen gland complex, which adds little to organic weight due to its high concentration of calcium spherules (Catalán et al., 2006).

Based on SHG, RAE and RAW indices, females were relatively more globous with less expanded apertures but with a similar aperture width relative to males; this sexual dimorphism can also be summarized as differences in the width of the last whorl (total width – aperture width), which was higher in females. This pattern of sexual dimorphism was not affected by the trophic availability. So, although sexual dimorphism probably increases the overall morphological variability of the shell and causes difficulties in taxonomic identification (Cowie, 2002), this effect is not worsened by interactions with food availability.

Previous researchers found sexual dimorphism in field collected adult snails of *P. canaliculata*, specifically in the opercular width to length ratio (Cazzaniga, 1990) and in the aperture width to shell length ratio (Estebenet, 1998). However, they did not find any differences in shell globosity, a sexually dimorphic variable in the present study, whereas we did not find any sexual dimorphism in shell aperture shape (as shown in the RAS index). These authors worked with lentic populations (two different ponds located near to the La Plata river, about 450 km to the NE of our study site), while in the present study the snails came from a lotic population in the Curamalal Grande stream, which belongs to a closed drainage basin (Encadenadas del Oeste basin; Martín et al., 2001), and hence this inter-population variation in sexual dimorphism could be due both to environmental and genetic differences. On the other hand, the differences with the previous studies may be related to the size of the snails analyzed. The males studied here were in general small to medium in size (24 to 40 mm; Tamburi & Martín, 2009a), while in previous studies snails had been obtained from the field and mostly large sizes had been selected: all the males were between 39 and 54 mm in Cazzaniga's study (1990) and 80% between 40 and 60 mm in the study by Estebenet (1998). Probably aperture-related variables are better for differentiating sex in large snails in which the development of the penis sheath deformed the aperture to a greater extent, while our criterion for sex determination did not need snails near to their asymptotic size.

The organic density, a somatic index interpretable as a nutritional or condition parameter, can be an adequate indicator of actual trophic availability in the field, although it must be estimated separately for males and females. Condition indices used frequently in marine mollusks use the relationship between body weight and shell weight (or total weight including the shell) to determine nutritional state (e.g., De la Hoz Aristizábal, 2010; Mouneyrac et al., 2008). However, in freshwater environments, water calcium and pH constitute strong sources of interpopulation variation in shell weight (e.g., in *Pomacea paludosa* (Say 1829); Glass & Darby, 2008) that have potential confounding effects on the interpretation of such indices. Condition indices, including shell weight, can also be affected by preservation procedures, especially when shell weight is estimated

indirectly (Martín, 2001). The organic density, calculated here as the ratio of body weight to shell volume, avoids this source of variation associated with shell weight, allowing better interpopulation comparisons of nutritional state. Furthermore, our results for this variable indicate that the meat profit obtained by such specialized predators as the snail kite *Rostrhamus sociabilis* (Vieillot, 1987) (see Snyder & Snyder, 1969; Sykes et al., 1995) from an apple snail of a given volume would be lower in habitats with low trophic availability (from the snail's point of view).

The euryoecius character of *P. canaliculata* indicates that the presence of fossil shells is probably not a good indicator of past habitat types (Martín & De Francesco, 2006), although the plasticity of this apple snail indicates that other traits would be useful in paleo-environmental reconstruction. The overall shell density (OSD) and the relative aperture width (RAW) can be used as paleo-environmental indicators of productivity, as they show a significant effect of food availability and can be measured on empty shells of *P. canaliculata*. However, the relative shell thickness of *Pomacea* probably undergoes changes due to the water pH and calcium concentration (Glass & Darby, 2008) that may hamper the interpretation. Another factor that must be considered is that some part of the morphological variation among populations may have a genetic component (Estebenet & Martín, 2003).

On the whole our study showed, for the first time for an apple snail, the multiple and diverse effects of a particular environmental factor on morphometric and somatic indices. Food availability, the factor selected in our study, has also been found to produce plastic responses in life history traits (e.g., age and size at maturity or fecundity) of *P. canaliculata* (Estoy et al., 2002a, b; Tamburi & Martín, 2009a, b, 2011). Some of this reaction norms to food availability have been considered as adaptive for a snail living in environments where trophic resources are highly variable and unpredictable (Tamburi & Martín, 2009a, 2011). None of the effects of food availability showed here on morphometric and somatic indices are clearly interpretable as an adaptive developmental plasticity. Instead, most of them are probably the collateral outcome of physiological changes that are either adaptive or strongly influenced by the environment, or a combination of both.

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LONGEVITY AND GROWTH RATES OF THE GUMBOOT CHITON,
CRYPTOCHITON STELLERI, AND THE BLACK LEATHER CHITON,
KATHARINA TUNICATA

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ABSTRACT

Few studies have documented chiton growth rates, and lifespans for most chiton species remain unknown. This study presents the first size-at-age and longevity estimates for *Cryptochiton stelleri* and *Katharina tunicata*, two of the largest known species of chitons. Age estimates were based on growth ring counts obtained by cross-sectioning and polishing valves. This process alone showed clear growth rings in shell plates of *K. tunicata*. Valves of *C. stelleri* did not show conspicuous growth rings in cross section, so the acetate peel technique was used to highlight growth bands. Linear growth in terms of body length and shell width approached an asymptote in both species and was described with the von Bertalanffy growth function. Both *C. stelleri* and *K. tunicata* displayed indeterminate growth with regard to tissue weight and shell weight, continuing to maintain a steady growth rate throughout their lifespans. The oldest *K. tunicata* specimen was estimated to be 17 years old. The oldest *C. stelleri* was estimated to be 40 years old, making it the longest-lived chiton species known.

Key words: mollusk, age, lifespan, sclerochronology, growth.

INTRODUCTION

Growth rate is one of the most fundamental life history parameters and is critical in developing models of the overall health, structure, and reproductive output of a population. Comprehensive knowledge of population structure is vital to the understanding of population dynamics for any species, especially those that are long-lived, because size and longevity impact the lifetime reproductive output of an individual or population (O'Farrell & Botsford, 2005). Growth rate information is available for many marine organisms, especially those with commercial value, such as fish and bivalves (Gang et al., 2008; Abele et al., 2009). Despite the relative abundance of growth rate data in general, established growth curves for some marine invertebrates are less common. This is particularly true for the chitons, class Polyplacophora.

The gumboot chiton, *Cryptochiton stelleri* (Middendorff, 1846), and the black leather chiton, *Katharina tunicata* (Wood, 1815), are fairly common inhabitants of the intertidal zone throughout their shared range (Japan and Kamchatka to Alaska and down the Pacific Northwest coast to central California) (Him-

melman, 1978; Yates, 1989). It is well-known that *C. stelleri* is the largest species of chiton in the world, at up to 36 cm long and up to 2,000 g (Lord, 2011b), and it feeds on a variety of seaweeds, including the algal genera *Cryptopleura*, *Mazzaella*, *Ulva*, *Nereocystis*, and *Odonthalia* (Yates, 1989; DeBusk et al., 2000). Like *C. stelleri*, *K. tunicata* feeds largely on macroalgae, in this case the kelp *Saccharina sessilis* and occasionally erect coralline algae or other leafy seaweed (Dethier & Duggins, 1984). *Katharina tunicata* can reach a length of up to 12 cm, making it the second largest chiton (to *C. stelleri*) in the northeastern Pacific (Himmelman, 1978). The only published study on the age or growth of *K. tunicata* was done by Heath (1905), who estimated that this species could live for three years in central California. The same study estimated *C. stelleri* lifespan at four years, but later studies have suggested that this chiton may live considerably longer.

Multiple studies have focused on the growth rate of *C. stelleri*, but few conclusions have been reached. Studies by MacGinitie & MacGinitie (1968) and Palmer & Frank (1974) attempted to use growth lines on the outside of the valves to determine age of this species, but the researchers ultimately decided that

these lines were too obscure to use for aging purposes. Despite the inability to fully interpret the growth lines, a number of studies have suggested that *C. stelleri* is quite long-lived, living up to 20 years or more (MacGinitie & MacGinitie, 1968; Palmer & Frank, 1974; Yates, 1989). However, Palmer & Frank (1974) expressed doubts about the external growth ring method used by MacGinitie & MacGinitie (1968) to arrive at the estimate of 20 years. Lord (2011a) documented the juvenile growth rate of *C. stelleri*, but did not attempt to describe adult growth. As such, little is known about the growth and longevity of *C. stelleri* and even less about the growth patterns of *K. tunicata*. This is partly due to the flexible morphology of chitons (especially *C. stelleri* and *K. tunicata*), which makes measurements of length highly variable.

The present study closely examined the ontogeny and growth rates of *C. stelleri* and used the growth rates of juveniles and young individuals to support age estimates based on growth rings. It was hypothesized that internal shell growth rings could be used to approximate age in *C. stelleri* and *K. tunicata* and would reveal longer lifespans than previously reported, given the large size of these chitons. A wide range of morphological measurements were used to facilitate the first detailed description of growth for the largest chitons in the world, *Cryptochiton stelleri* and *Katharina tunicata*.

MATERIALS AND METHODS

This study was conducted primarily along the central Oregon coast. *Katharina tunicata* specimens (n = 40) that were measured and sacrificed were taken from South Cove, Cape Arago, Oregon, ten km south of Charleston, Oregon (43°18.191'N, 124°23.198'W). *Cryptochiton stelleri* individuals that were sacrificed in order to measure the growth rings in their valves were taken from Lighthouse Island, Sunset Bay, and Cape Arago, all near Charleston, Oregon. Only nine *C. stelleri* were taken from each site in January 2010 in order to minimize collecting impact. The majority of the valves that were used to analyze growth lines were from individuals that washed up dead on the beach, so were not killed for the purpose of this study. In February 2009, 32 dead *C. stelleri* were collected from a beach near Fort Ebey State Park, Washington, and 12 individuals were collected from the south end of Asilomar Beach near Monterey, California in December 2009.

Cryptochiton stelleri that were washed up on the beaches in Washington and California were too deteriorated to obtain any body size information, so all external body measurements were done solely on individuals from the sites in Oregon. All size and age data for *K. tunicata* were from the specimens collected at the south cove of Cape Arago in Charleston, Oregon. The *C. stelleri* shell data were pooled from all sites on the southern Oregon coast (10 km range) and were analyzed separately from the shells of the specimens collected near Fort Ebey, Washington, and Monterey, California. External measurements included body length (*K. tunicata*) and circumference when curled up in a ball (*C. stelleri*). Body length of *K. tunicata* was measured along the longest axis, from the front to the back of the girdle when the specimens were relaxed, with an accuracy of 1 mm.

Circumference was used for *C. stelleri* because of its flexible morphology and was determined by first picking up and holding specimens until they were tightly curled and then measuring the circumference longitudinally to the nearest millimeter. The reliability of this method was verified by making repeated measurements of 15 specimens, which were given time to uncurl between each measurement. Volume (water displacement) and weight in air were also measured for *C. stelleri* and *K. tunicata*. All specimens were kept in a flowing seawater table for a week prior to measurement so that weight and volume measures would not be biased by environmental factors. During this time, seaweeds *Mazzaella splendens* and *Sacharina sessilis* were fed to *C. stelleri* and *K. tunicata*, respectively.

Specimens were relaxed, then killed with a 7.5% magnesium chloride solution the same day external measurements were taken and one week after field collection. Valves were removed, cleaned and dried for two weeks, weighed on a digital scale and then photographed and measured digitally using ImageJ (available at <http://rsb.info.nih.gov/ij>). The valve measurements, length (at the shortest point) and width (at the widest point), were made on the third, fourth and fifth valves for each *Katharina tunicata* specimen (Fig. 1). Using multiple valves per specimen improved the reliability of the growth ring counts for each individual, and these three valves were the largest and easiest to handle for *K. tunicata*. The same length, width and weight measurements were made on all intact valves from California, Oregon, and Washington *Cryptochiton stelleri*, for a total of over 300 valves measured and weighed from

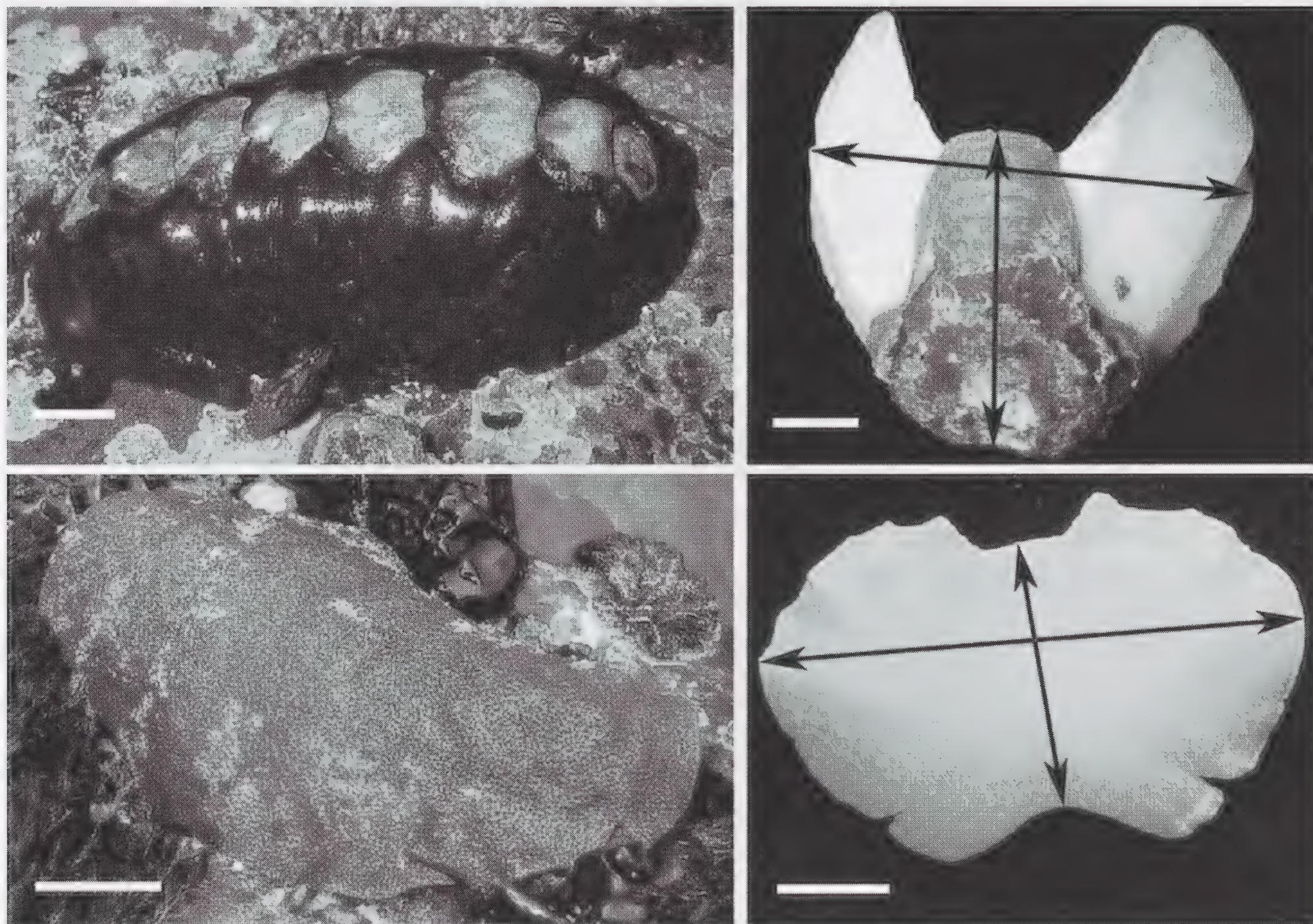


FIG. 1. Dorsal views of *Katharina tunicata* (upper left, scale = 1 cm) and *Cryptochiton stelleri* (lower left, scale = 5 cm) in the intertidal zone in central Oregon. Top-down views *K. tunicata* (upper right, scale = 5 mm) and *C. stelleri* (lower right, scale = 1 cm) valves are pictured after dissection and cleaning. Valves were cross-sectioned along the axes indicated by arrows.

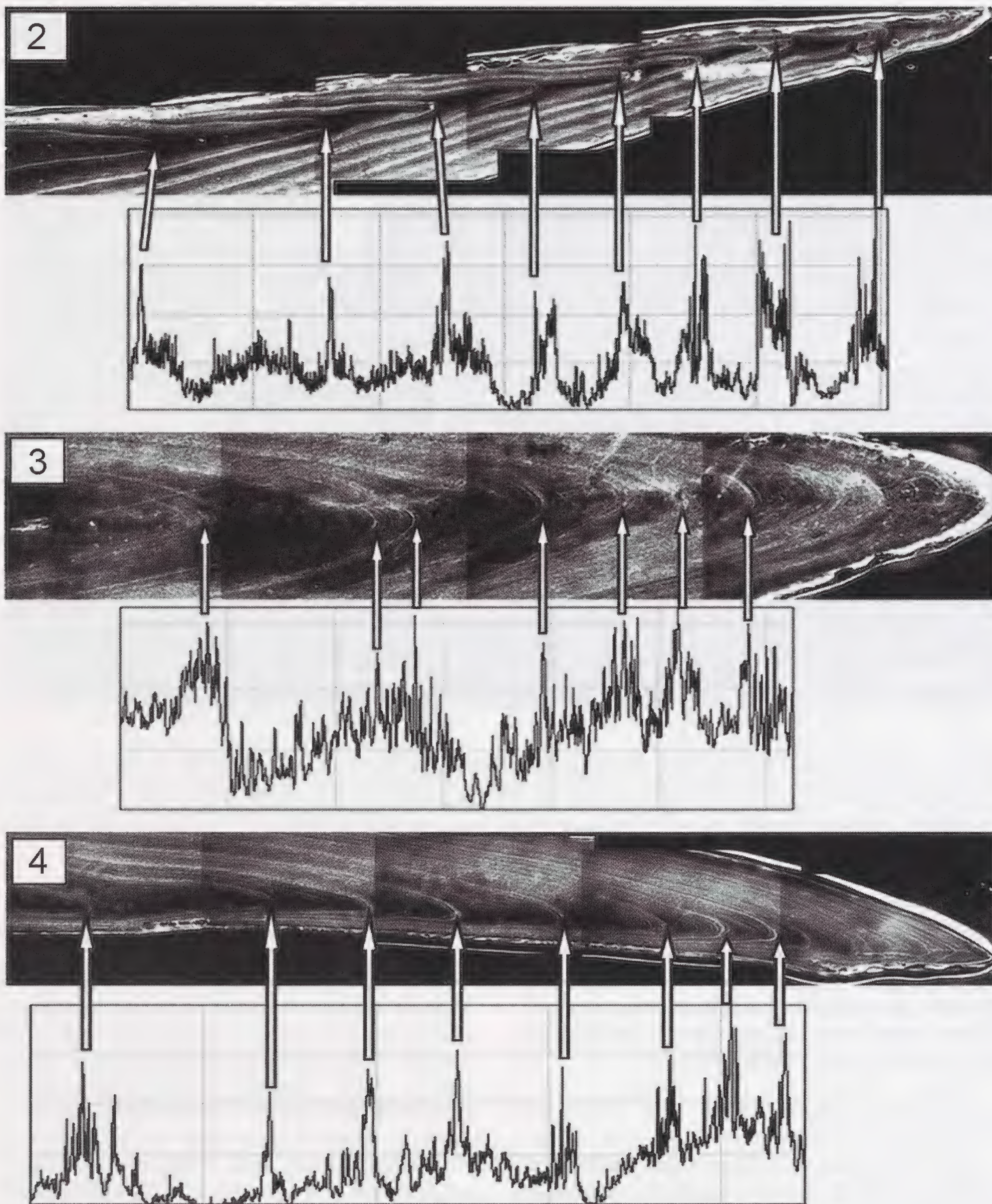
71 individuals. Because many of the Washington and California specimens that had washed up in the intertidal zone had several broken valves (approximately 58% of valves were broken) (excluded from analysis), three specific valves could not be used for each specimen, so all intact valves were included.

Growth Ring Counts

Valves were cross-sectioned using a Dremel® rotary tool with a diamond-coated cutting blade in order to observe growth rings. For each *C. stelleri* specimen, all valves were cut into quarter sections, on the long and short axes of the valve. Barring breakage, all sections of all valves were examined to assess growth rings (mean = 6 valves per specimen). Several of the dead, washed up specimens had broken valves that were unusable or which could only be measured in one direction. *Katharina tunicata* valves (valves 3, 4, 5) were also cut into quarter sections along the long and short axes.

Valves of both species were then polished with increasingly fine silicone carbide grinding paper, with a final grit of 1,200.

Clear, dark growth rings were visible on *K. tunicata* valves with the naked eye after cross-sectioning and polishing (Fig. 5) and were counted under a dissecting microscope. Major growth rings in *C. stelleri* were not visible under a dissecting microscope so were examined via acetate peels following the protocol of Black et al. (2009). Acetate peels were taped to microscope slides and examined for growth rings on a compound light microscope. The distances between valve rings were measured using an eyepiece micrometer, and photos were taken of acetate peels using an Optixcam Summit 5.0 series® digital microscope camera. Photographs of valves were analyzed for the density of valve lines using the plot profile function in ImageJ in order to compare the growth of *C. stelleri* individuals from California, Oregon, and Washington. This was done by making transects down the middle of each valve and



FIGS. 2–4. Photographs of acetate peels of sectioned *C. stelleri* valves from Washington, California, and Oregon. Graph shows associated plots of gray values from ImageJ, with peaks in gray value associated with each growth line because of the brightness of the line compared to the dark region of growth in between lines. Valves and graphs were chosen as representative examples of the differences in banding in the valves of Washington, Oregon and California specimens. FIG. 2: Acetate peel of valve cross-section from Washington. Growth lines are clearly defined, with very few smaller growth rings between major rings; FIG. 3: Acetate peel of valve cross-section from California. Growth lines are more obscure, with many pronounced smaller lines in between each growth band; FIG. 4: Acetate peel of valve cross section from Oregon. Growth lines are more clearly defined than California, but less clear than in individuals from Washington.

TABLE 1. All equations shown are the curves that were fit to size and age data for *Cryptochiton stelleri* and *Katharina tunicata*. Functions are shown that describe both the overall tissue growth and the shell (valve) growth of each species.

Species	y	x	Function	Equation	R ²
<i>Katharina tunicata</i>	Body Length (mm)		Von Bertalanffy	$y = 114 * (1 - e^{-0.217x})$	0.84
	Volume (mL)		Linear	$y = 7.83x - 10.6$	0.84
<i>Cryptochiton stelleri</i>	Circumference (cm)		Von Bertalanffy	$y = 39.4 * (1 - e^{-0.702x})$	0.97
	Volume (mL)	Age	Linear	$y = 32.1x - 79.4$	0.91
<i>Katharina tunicata</i> valves	Valve 4 Width (mm)		Von Bertalanffy	$y = 33.4 * (1 - e^{-0.172x})$	0.93
	Valve 4 Weight (g)		Power (2 param.)	$y = 0.167 * x^{1.20}$	0.83
<i>Cryptochiton stelleri</i> valves	Valve 8 Width (mm)		Von Bertalanffy	$y = 31.9 * (1 - e^{-0.0681x})$	0.72
	Valve 8 Weight (g)		Power (2 param.)	$y = 0.154 * x^{1.32}$	0.80

analyzing the gray values along this transect. The plot profile function plots gray values on a scale from zero (black) to 255 (white), with peaks in gray value indicative of a major growth ring (Figs. 2–4).

Growth lines faded out towards the center of the valves on older *C. stelleri* specimens, so an additive process was used to estimate the number of rings for these individuals. Since smaller specimens had all of their growth rings visible all the way to the center, the distance between each ring and the next was measured and a dataset was compiled that included the number of rings present at each distance from the center of the valve. These data were used (Figs. 6–8) to estimate the number of inner growth rings for those older individuals with growth rings that faded out towards the center of the valve. Growth increments were verified for *C. stelleri* using the list year technique described by Black et al. (2008), with conspicuously wide or narrow increments recorded and matched up between individuals of the same population.

Some *K. tunicata* individuals sampled were young enough (two growth rings) that there was not a large gap in the early part of the size-at-age curve for this species. However, no *C. stelleri* collected from any sites had fewer than four growth rings, leaving a gap in the early part of the size-at-age curve. Additional growth rate data for juvenile and young *C. stelleri* were used from Lord (2011b), who reported laboratory growth rates for juveniles of this species.

Size-at-Age Curves

Juvenile measurements of *C. stelleri* size at known age (Lord, 2011b) were combined with ring count information to create size-at-age

curves showing the changes in several different morphological characteristics with age. Growth rings in *K. tunicata* appeared as conspicuous opaque bands, making growth ring counting much simpler, since acetate peels were not needed to discern the growth lines.

Growth data were plotted and fitted with appropriate curves in Sigmaplot® for both *C. stelleri* and *K. tunicata* (derived equations shown in Table 1). In order to create a complete size-at-age curve, data for *C. stelleri* specimens from Oregon sites were combined because they showed similar patterns. Separate size-at-age curves were created for *K. tunicata* from Oregon and *C. stelleri* from Washington, Oregon, and California.

RESULTS

No differences were found in valve growth rates of *C. stelleri* between Washington, Oregon, and California, although there were differences in the shell deposition patterns at these different locations. Growth ring counts estimated the lifespan of *Katharina tunicata* as at least 17 years, and *Cryptochiton stelleri* as at least 40 years. Both *K. tunicata* and *C. stelleri* displayed continuous growth in terms of age, volume, weight, and valve weight. Growth for both species in terms of body length and shell width slowed with age and approached an asymptote.

Growth Ring Counts

Acetate peels of *C. stelleri* valves showed clear major growth lines as bright (opaque) rings separated by dark (transparent) incre-

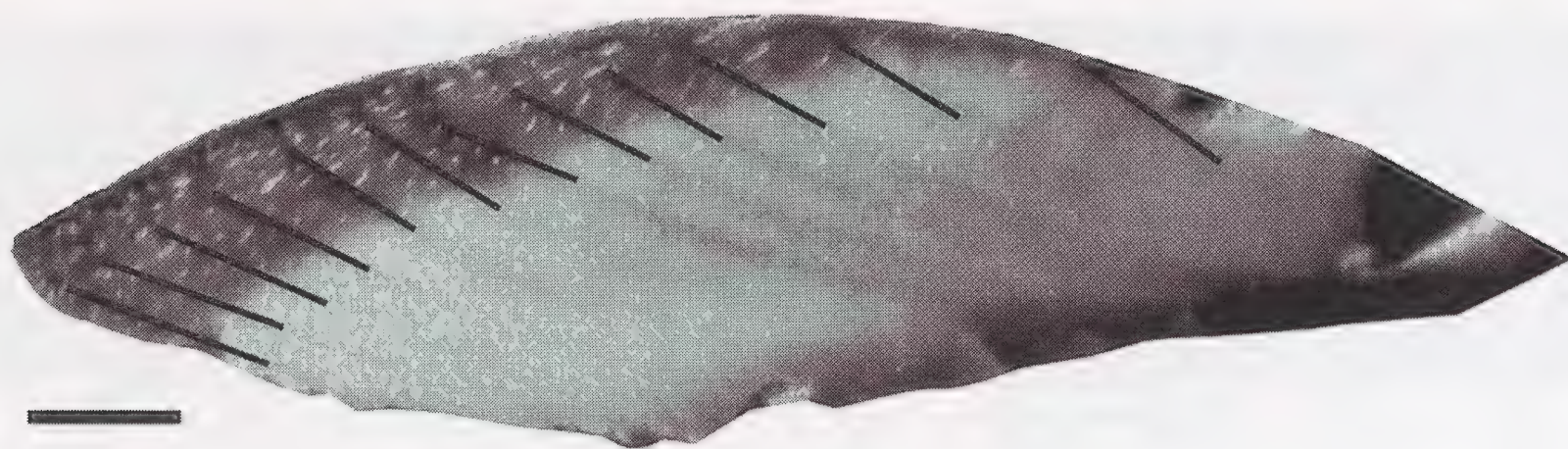


FIG. 5. Photograph of *K. tunicata* valve under a dissecting microscope (scale = 1 mm). Valve was cross-sectioned vertically (shown in Fig. 1) and then examined for major growth lines, which are each highlighted by overlaid black lines in the image.

ments of faster growth (Figs. 2–4). Spikes or peaks in the gray value in ImageJ coincided with major growth lines (Figs. 2–4) and helped to discern major growth rings. For *K. tunicata*, only major rings were visible under the dissecting microscope, so discerning between major and minor rings was not an issue. The additive method for counting rings (previously described) was fairly precise, as the curves estimating the numbers of growth rings to the center had a 95% confidence interval of approximately ± 2 rings (Figs. 6–8). Individual *C. stelleri* matched an average of 82% of the markers (conspicuously wide or narrow growth bands) selected for comparison by the list year technique. This ensured that the increments were aligned and made it more likely that age predictions were accurate across all specimens.

Cryptochiton stelleri valves from Fort Ebey, Washington (Fig. 2) showed very distinct growth rings, with very few smaller growth lines between the major annual bands. The major growth rings on the valves from Asilomar, California (Fig. 3), were much less pronounced and were obscured by a large number smaller growth rings clumped around each major ring. This distinction between Washington and California is clear on both the acetate peels and the gray value graphs. Valves from Oregon (Fig. 4) showed distinct growth lines similar to those from Washington, but slightly less defined.

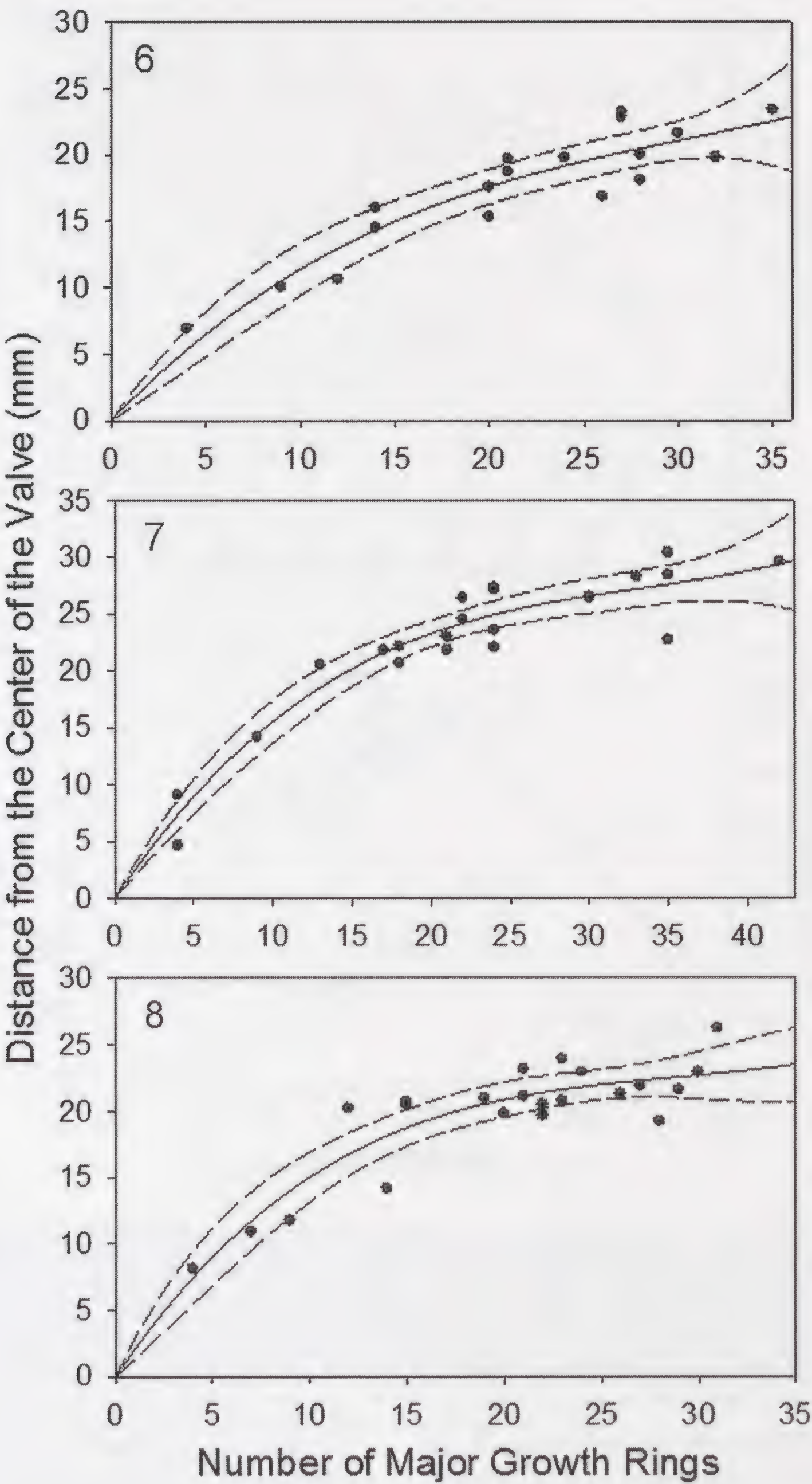
There were no significant differences in spacing between major growth rings in valves from the three different states ($p > 0.3$). However, there were differences (one way ANOVA, $df = 110$, $F = 15.39$, $p < 0.0001$) in the dispersion of smaller growth rings around each major growth band. Each growth band is made up of a series of small (subannual) rings that form “bands” when they are spaced very closely together, indicative of periods of slow growth

(winter). Both Washington (Tukey’s HSD, $p < 0.01$) and Oregon (Tukey’s HSD, $p < 0.01$) had growth lines that were significantly more tightly clustered (width of peak in gray value) than in the California valves. These tightly clustered minor growth lines made major growth bands in Washington and Oregon valves very distinct. Valves from Oregon *C. stelleri* specimens were not significantly different from Washington valves with regard to the clustering (of small growth marks around major growth rings (t-test, $p > 0.2$).

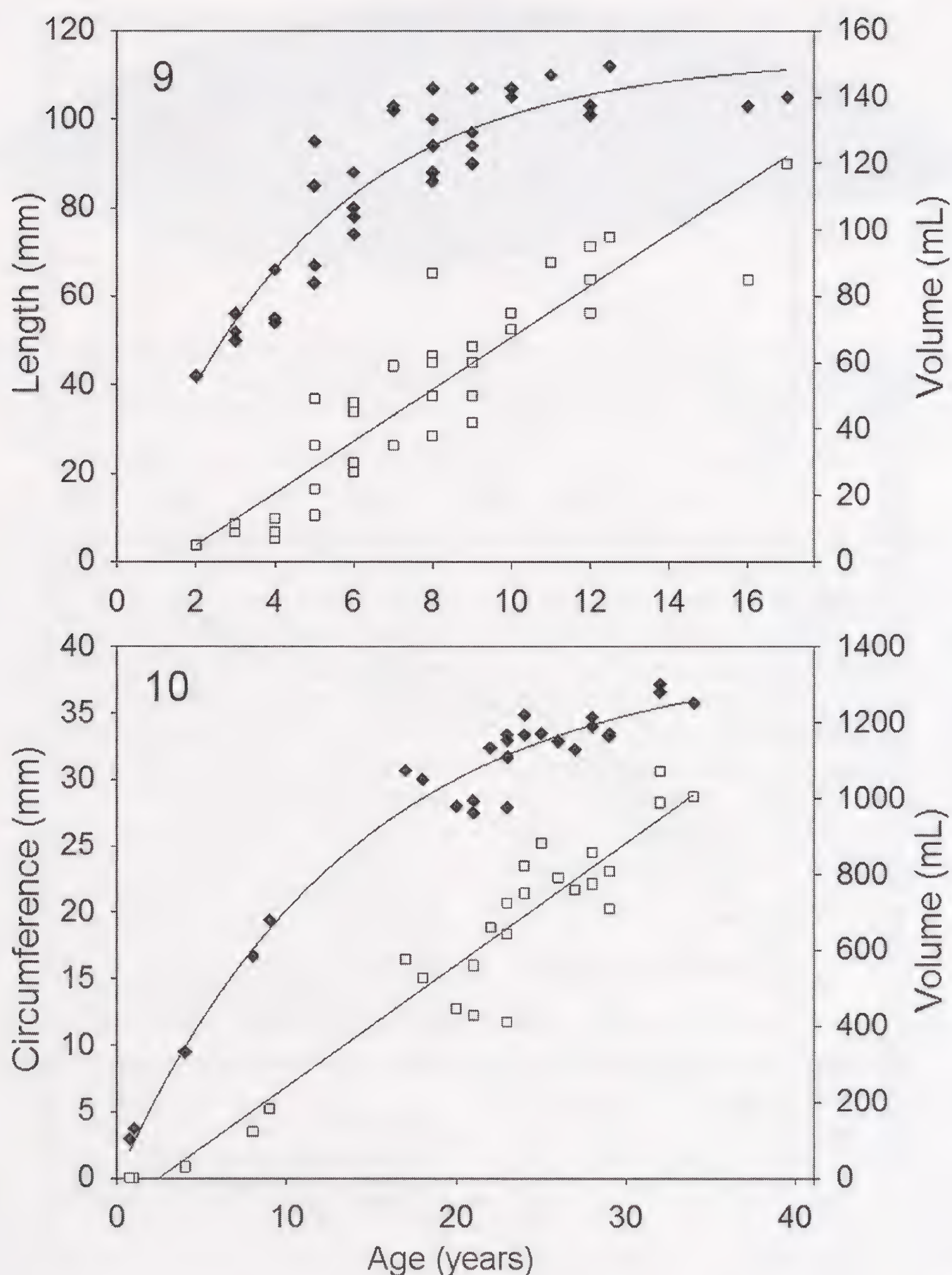
Size-at-Age Curves

Counts of the major growth rings were used to create size-at-age curves for *C. stelleri* and *K. tunicata*. Using ages inferred from the growth rings, body circumference of *C. stelleri* and length of *K. tunicata* were plotted against age, with juvenile *C. stelleri* data included from Lord (2011b) (Figs. 9, 10). The circumference-age or length-age data for both species were fit well ($r^2 = 0.84$ for *K. tunicata*, 0.97 for *C. stelleri*) (Table 1) by the von Bertalanffy growth function (von Bertalanffy, 1938), which is commonly used to describe growth of intertidal organisms (e.g., Ebert & Russell, 1992; Steffani & Branch, 2003) and is shown here: $L_t = L_\infty (1 - e^{-kt})$. L_t is body length, L_∞ is maximum or asymptotic body length, k is the instantaneous growth rate, and t is age.

Body volume was also plotted against estimated age, producing a different shaped curve for both species than the curves based on length and circumference (Figs. 9, 10). In both species, size, as measured by volume, increased continuously with age and was fit with a linear function ($r^2 = 0.84$ for *K. tunicata*, 0.91 for *C. stelleri*) (Table 1). Valve growth showed similar patterns (Figs. 11, 12), as valve weight growth rates actually increased with age for both spe-



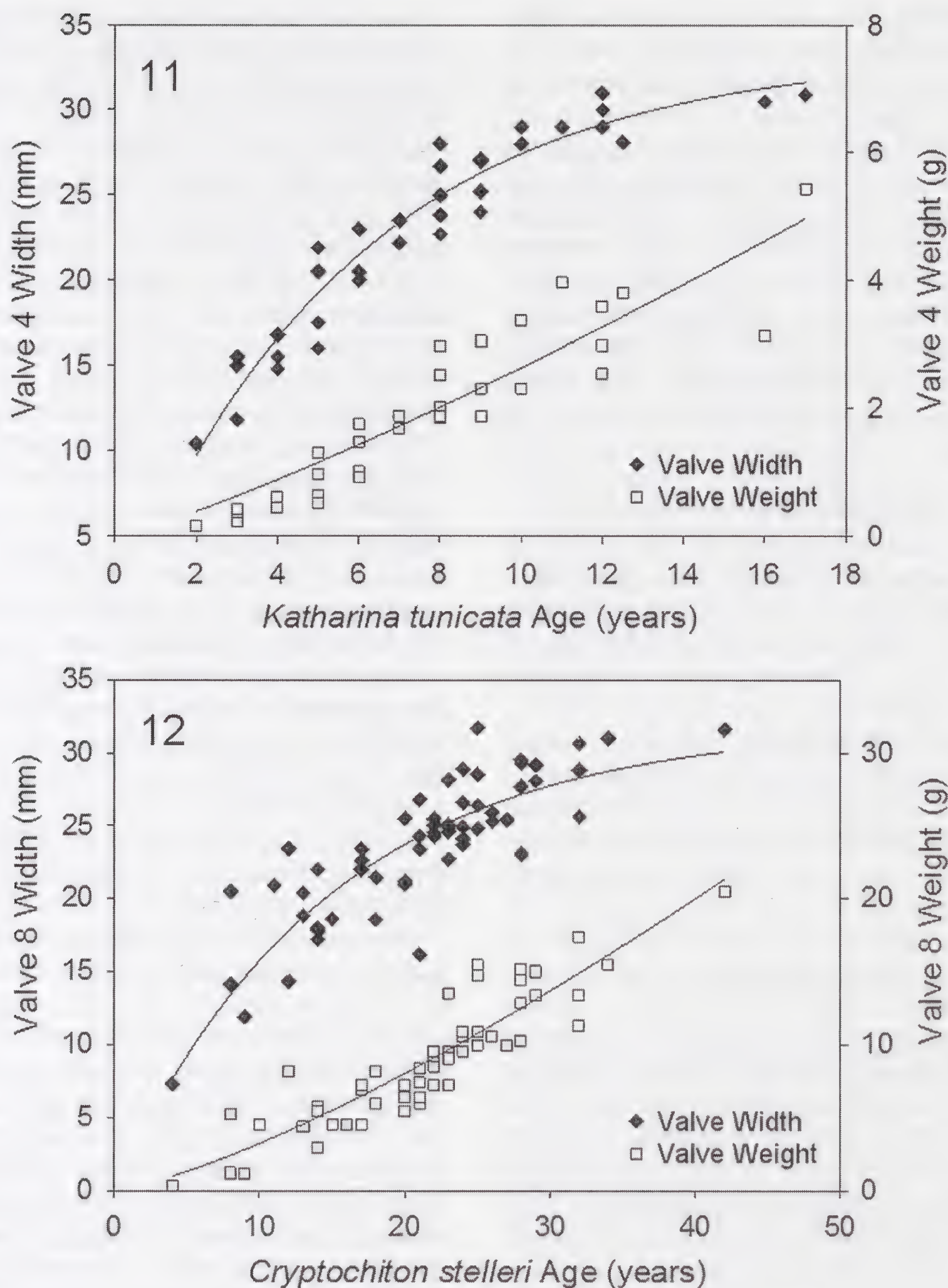
FIGS. 6–8. Curves used to help estimate the number of *C. stelleri* growth rings when valves had growth rings that faded towards the center. These curves were used to predict the number of rings that would be in that distance to the center of the valve. Data were fit with three parameter polynomial functions to describe patterns. 95% confidence intervals are shown on each graph. FIG. 6: Valve 1; FIG. 7: Valve 7; FIG. 8: Valve 8.



FIGS. 9, 10. Size-at-age curves based on volume and length (*K. tunicata*) or circumference (*C. stelleri*). Body length and body volume are both plotted against presumptive annual growth rings. Data are fit with growth functions described in Table 1. FIG. 9: Size-at-age curves for *K. tunicata* ($n = 35$); FIG. 10: Size-at-age curves for *C. stelleri* ($n = 27$). Circumference is used as a linear measure of size instead of length because of the flexibility of the organism.

cies and were fit with two-parameter power functions ($r^2 = 0.83$ for *K. tunicata*, 0.80 for *C. stelleri*) (Table 1). Valve width growth slowed with age in both species and was fit with the von Bertalanffy growth function ($r^2 = 0.93$ for *K. tunicata*, 0.72 for *C. stelleri*) (Table 1). These data included the datasets from Washington and California *C. stelleri* specimens, since they showed similar growth patterns to those from Oregon.

The size-at-age curves created for *K. tunicata* estimate that the oldest specimen sampled in this study was 17 years old. For *C. stelleri*, the oldest specimen sampled was approximately 42 years old. Both species have maximum recorded sizes larger than those used in the this study, so these lifespan estimates are likely underestimates of the longest possible lifespan for these species.



FIGS. 11, 12. Size-at-age curve for age and valve width and valve weight data for combined Washington, Oregon and California specimens of *C. stelleri* and Oregon specimens of *K. tunicata*. For both species, the valve width growth rate slows with age, while the valve weight growth rate increases with age. Data were fit with functions given in Table 1. FIG. 11: Size-at-age curve for *C. stelleri* valve 8 width and valve 8 weight plotted against presumptive annual growth rings; FIG. 12: Size-at-age curve for *K. tunicata* valve 4 width and valve 4 weight plotted against presumptive annual growth rings.

DISCUSSION

Several methods have been used to measure chiton growth, including measurements of valve width (Soliman et al., 1996), body length (Glynn, 1970), body weight (Palmer & Frank, 1974), external shell markings (Crozier,

1918; MacGinitie & MacGinitie, 1968) and internal growth rings (Jones & Crisp, 1985). Previous studies on the life histories of *Cryptochiton stelleri* and *Katharina tunicata* have been inconclusive regarding the growth rates and lifespans of these large chitons (Heath, 1905; MacGinitie & MacGinitie, 1968; Palmer

& Frank, 1974). The analysis of internal shell growth lines in this study resulted in the first accurate description of growth patterns for *C. stelleri* and *K. tunicata*.

Sclerochronology is becoming commonplace in growth studies of hard-shelled mollusks, especially bivalves (e.g., Schone, 2003; Black et al., 2008). Some of the difficulties involved in using this method of age determination are in confirming the assumption that growth rings are deposited regularly and determining the time interval between growth lines. In addition, it can be difficult to discern individual growth lines, making this process potentially subjective (Campana, 2001). Distinguishing between major growth lines was not difficult in the present study because of the clarity of these lines, with large transparent gaps in-between opaque rings (Figs. 2–4). Major growth lines were clearly visible in polished valve cross-sections for *K. tunicata* (Fig. 5) and in acetate peels for *C. stelleri*.

Growth increments in the valves of *K. tunicata* and *C. stelleri* were laid down at regular intervals, as evidenced by the clear distinction between periods of slow and fast growth. The use of several valves from the same individual to confirm growth ring counts also indicated regular increments of shell deposition. This was further confirmed by the list-year technique (Black et al., 2008) with *C. stelleri*, which matched conspicuously wide and narrow growth increments between individuals and between valves of the same individual. The matching of 82% of these markers across all specimens not only verified age estimates but also showed population-wide patterns of shell deposition that would likely not occur if growth rings were deposited inconsistently.

In order for growth ring counts to be reliably converted to age estimates and used to create growth curves, it was necessary to establish annual shell deposition. Annual growth rings are commonly formed during winter, when a slowdown in growth can result from gonad production, colder temperatures, increased storms, and lower food availability (Crozier, 1918; Baxter & Jones, 1978; Brousseau, 1979; MacDonald & Thomas, 1980; Picken, 1980; Thompson et al., 1980). The production of annual growth rings is especially common among organisms with annual food supplies, such as phytoplankton or macroalgae. *Cryptochiton stelleri* and *Katharina tunicata* spawn once per year in the spring (Giese et al., 1959; Tucker & Giese, 1962; Himmelman, 1978; Yates, 1989) and feed predominantly on seasonally abun-

dant macroalgae (Dethier & Duggins, 1984; Yates, 1989), so it is likely that both species have a lower food intake in the winter and allocate most energy to gonad production. Therefore, shell growth should be minimal during this time, resulting in tightly clustered minor growth lines that appear as well-defined major growth bands deposited annually in the winter.

The finding that *Cryptochiton stelleri* valves between the sites in Washington, Oregon, and California on the west coast of the United States showed no significant differences in growth ring spacing indicates similar growth rates between these three locations. This suggests that shell deposition (and volumetric growth, by proxy) does not vary with latitude through this subset of the range of *C. stelleri*. However, the inclusion of only three locations precludes the ability to draw wide-scale latitudinal conclusions based on the present study. In addition, the 1,600 kilometer distance between the southern (California) and northern (Washington) sites are still only a small segment of the range of this species. Since the range of *C. stelleri* extends as far north as the Aleutian Islands and Kamchatka, it may display altered growth patterns in the different climactic regimes that it inhabits.

Some variation in *C. stelleri* shell deposition with latitude was evident in the difference found in the dispersion of smaller (minor) growth rings around major annual rings in the California valves compared to those from Washington and Oregon. Since major shell growth bands are collections of tightly spaced smaller minor growth lines, more widely dispersed minor lines, such as those found on California valves, indicate less seasonal differences in growth. This is supported by seasonal patterns in macroalgal abundance, since such macroalgae as *Mazzaella splendens* (a preferred food of *C. stelleri*) are seasonal in Washington and Oregon but present year-round in Monterey, California (Dyck & DeWreede, 2006). While *C. stelleri* can go months without feeding in Oregon where macroalgae is largely seasonal (Yates, 1989), it is likely that this species feeds year-round in California, resulting in the less-defined major growth bands. Since the acetate peel technique and multiple sites were not used for *K. tunicata*, minor growth lines were not analyzed, but similar latitudinal patterns would be expected based on range and feeding habits.

The presumptive conclusion that growth rings in *C. stelleri* and *K. tunicata* are annual allowed for the creation of size-at-age curves

and longevity estimates for these species. In terms of length and shell width, both species displayed asymptotic growth patterns, which are commonly described by the von Bertalanffy growth function (Figs. 9–12). The length of *K. tunicata* (Fig. 9) leveled off more with age than did *C. stelleri* circumference (Fig. 10), but this may be a result of the size and age of specimens included in this study. The largest *K. tunicata* in this study was 11 cm long, compared to a maximum length of 12 cm for this species (Himmelman, 1978). In contrast, the longest *C. stelleri* included was approximately 25 cm, compared to a species maximum of 36 cm. Therefore, *C. stelleri* data may have shown the same pattern of minimal linear growth if larger individuals were included. In terms of volume and shell weight, growth was continuous or even increasing with age for both species (Figs. 9–12). It was clearly possible for both species of chiton to show increases in overall growth rate and shell deposition even as linear growth (shell width, body length) slowed down. These differing patterns are expected because volume generally varies as the cube of length, but it is interesting to note that both *C. stelleri* and *K. tunicata* do not slow down their volumetric growth rate even at advanced ages. This could be an adaptation to allow for increased feeding or reproductive output with age, but requires further study.

The longevity estimates for both *C. stelleri* and *K. tunicata* were much longer than previously reported by Heath (1905) or other studies in the case of *C. stelleri* (MacGinitie & MacGinitie, 1968; Palmer & Frank, 1974). The study by Heath (1905) was a largely anecdotal report that did not quantitatively assess growth rates of these species but instead made estimates based on sizes observed in the field. Growth rates and lifespans determined in this fashion are often underestimates, since linear growth slows with age (Figs. 9–12), making differences of even five years nearly imperceptible without detailed analysis. It is typical for estimates of lifespans based on sclerochronology to exceed previous estimates, especially for slow growing species such as the ocean quahog *Arctica islandica* (Strahl et al., 2007) and the Pacific geoduck *Panopea abrupta* (Black et al., 2008). This is due to the level of detail that can be observed in close examination of growth lines deposited in the shells. Studies such as the one by MacGinitie & MacGinitie (1968) that use external growth lines rely on obscure contours that can be altered by shell deterioration and can mask more detailed patterns. The

difference between external growth lines and internal is illustrated by contrasting Figure 1 (external) with Figures 2–5 (internal), which show an immense level of detail, especially in the acetate peels of *C. stelleri* (Figs. 2–4). The use of internal growth lines for *Cryptochiton stelleri* and *Katharina tunicata* allowed for a detailed analysis of their growth patterns and thus served as a useful tool for age determination. Sclerochronology could be applied to further understanding annual and sub-annual patterns of shell deposition in *C. stelleri* and *K. tunicata* as well as other chiton species.

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EVOLUTION OF THE RADULAR APPARATUS IN CONOIDEA (GASTROPODA: NEOGASTROPODA) AS INFERRED FROM A MOLECULAR PHYLOGENY

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ABSTRACT

The anatomy and evolution of the radular apparatus in predatory marine gastropods of the superfamily Conoidea is reconstructed on the basis of a molecular phylogeny, based on three mitochondrial genes (COI, 12S and 16S) for 102 species. A unique feeding mechanism involving use of individual marginal radular teeth at the proboscis tip for stabbing and poisoning of prey is here assumed to appear at the earliest stages of evolution of the group. The initial major evolutionary event in Conoidea was the divergence to two main branches. One is characterized by mostly hypodermic marginal teeth and absence of an odontophore, while the other possesses a radula with primarily duplex marginal teeth, a strong subradular membrane and retains a fully functional odontophore. The radular types that have previously been considered most ancestral, “prototypic” for the group (flat marginal teeth; multicuspid lateral teeth of Drilliidae; solid recurved teeth of *Pseudomelatomina* and *Duplicaria*), were found to be derived conditions. Solid recurved teeth appeared twice, independently, in Conoidea – in Pseudomelatominae and Terebridae. The Terebridae, the sister group of Turridae, are characterized by very high radular variability, and the transformation of the marginal radular teeth within this single clade repeats the evolution of the radular apparatus across the entire Conoidea.

Key words: Conoidea, *Conus*, radula, molecular phylogeny, evolution, feeding mechanisms, morphological convergence, character mapping.

INTRODUCTION

Gastropods of the superfamily Conoidea (= Toxoglossa) constitute a hyperdiverse group of predatory marine snails that includes in particular the well-studied genus *Conus*. Conoideans are notable for the possession of a large venom gland (Figs. 1–4, vg), together with a highly modified radula.

An unusual peculiarity of Conoidea foregut anatomy is that the buccal mass with the radular sac is situated at the proboscis base (Fig. 1 – bm, rsod) and the radula cannot be protruded through the mouth and used for grabbing and rasping the prey.

The most outstanding character of Conoidea is the unique mechanism of envenomation of the prey. Some conoideans were long known to use individual teeth at the proboscis tip for stabbing and injecting neurotoxins into prey (e.g., Kohn, 1956). A marginal tooth is detached from

the subradular membrane, transferred to the proboscis tip (Figs. 2, 4), held by sphincter(s) in the buccal tube (Figs. 2, 4 – bts) and used for stabbing and envenomating the prey. Use of marginal teeth at the proboscis tip was observed directly and studied in detail in various species of *Conus* that possess elongate, barbed, harpoon-like, hollow marginal teeth (Kohn, 1956; Olivera et al., 1990; Kohn et al., 1999), through which the venom is injected into the prey (following Kantor & Taylor, 2000 we refer to these hollow marginal teeth as *hypodermic*). The prey is swallowed whole, sometimes being similar in size to the predator itself (e.g., Kantor, 2007). In these cases, the radular apparatus underwent profound transformation and the odontophore completely disappeared. Another important character is that the anterior part of the radular diverticulum, which is homologous to the sublingual pouch of other gastropods, is transformed into a caecum

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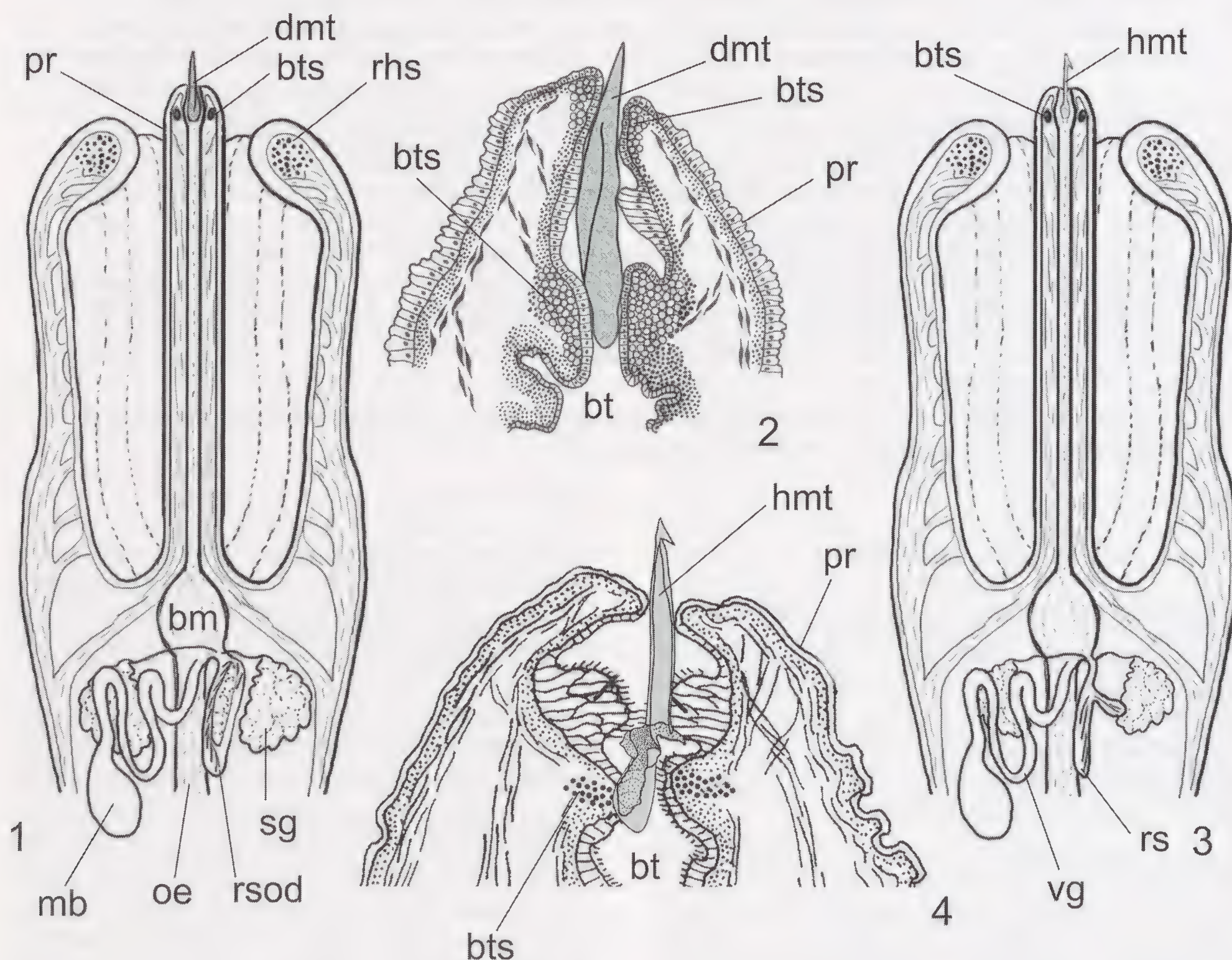
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("short-arm of the radular sac"), where fully formed marginal teeth are stored prior to their use at the proboscis tip (Taylor et al., 1993).

Conversely, in many conoideans the radular apparatus includes well-developed subradular membrane and a fully functional odontophore with muscles, thus suggesting that the radula still has some (although maybe limited) function as a complete organ. As in conoideans with hypodermic teeth, the radula and odontophore are situated at the proboscis base and normally cannot be protruded through the mouth (Fig.

1). In these conoideans, the marginal teeth can be of very different anatomy, but with few exceptions are not hollow, *non-hypodermic*. In conoideans with non-hypodermic marginal teeth (and a functional odontophore), a tooth separated from the rest of the radula was very often (in most preserved specimens examined) found held at the proboscis tip (Figs. 1, 2). Such teeth were first recorded in serial histological sections of proboscises in several species of *Aforia* (Cochlespiridae) (Sysoev & Kantor, 1987), Drilliidae (Sysoev & Kantor, 1989), and



FIGS. 1–4. Diagrammatic sections through the anterior foregut of Conoidea. FIG. 1: Anterior foregut of the Conoidea with non-hypodermic marginal radular teeth and odontophore (generalized representative of the clade B). A duplex marginal tooth detached from the subradular membrane is used at the proboscis tip for stabbing and envenomating the prey; FIG. 2: Section of the tip of the proboscis with the duplex marginal tooth held by sphincters of the buccal tube (actual specimen of *Aforia kupriyanovi* Sysoev & Kantor, 1988 – Cochlespiridae); FIG. 3: Anterior foregut of the Conoidea with hypodermic marginal radular teeth and lacking odontophore (generalized representative of clade A). A hypodermic marginal tooth detached from the subradular membrane is used at the proboscis tip; FIG. 4: Section of the tip of the proboscis with the hypodermic marginal tooth held by a sphincter of the buccal tube (actual specimen of *Phymorhynchus wareni* Sysoev & Kantor, 1995 – Raphitomidae). Abbreviations: bm – buccal mass; bt – buccal tube; bts – buccal tube sphincter; dmt – duplex marginal tooth at the proboscis tip; hmt – hypodermic marginal tooth at the proboscis tip; mb – muscular bulb of the venom gland; oe – oesophagus; pr – proboscis; rhs – rhynchostomal sphincter; rs – radular sac without odontophore; rsod – radular sac with odontophore; sg – salivary gland; vg – venom gland.

in three additional families, here referred to as Turridae, Clavatulidae and Pseudomelatomidae (Kantor & Taylor, 1991). The base of the tooth was held by special sphincter(s) and/or an epithelial pad of the buccal tube. Thus, the presence of marginal teeth detached from the radular membrane and of different morphologies, from solid duplex to specialized hypodermic, used one by one at the proboscis tip for stabbing the prey can be inferred from anatomical characters (presence of the sphincters in the buccal tube).

Therefore, the feeding mechanism of Conoidea involves use of single marginal tooth detached from the rest of the radula apparatus held at the proboscis tip for stabbing and poisoning the prey either through the central cavity of the hollow tooth (in hypodermic ones) or through the laceration made by the non-hypodermic tooth.

Peculiarities of the feeding have been discussed for different groups of Conoidea (e.g., Kantor & Sysoev, 1989; Taylor et al., 1993; Kantor et al., 1997; Kantor & Taylor, 2002), and several subtypes of feeding mechanisms have been suggested. Based on the foregut anatomy, the use of the teeth at the proboscis tip was found improbable in only two groups of radular bearing Conoidea that possess the proboscis – Strictispiridae, and the clade formed by the genera *Pseudomelatoma*, *Hormospira* and *Tiariturris*, previously recognized as a separate (sub)family Pseudomelatomidae (Kantor, 1988; Kantor & Taylor, 1991, 1994). In all others, the marginal teeth are used at the proboscis tip.

Despite the fact that the Conoidea are one of the most well-known groups of Neogastropoda from the point of view of anatomy and lately molecular phylogeny, data on their feeding and diet are still very limited. With the exception of *Conus*, information on diet is available for fewer than 50 species and involved much less direct observation (e.g., Shimek, 1983a–c, Heralde et al., 2010). Most of the conoideans (other than *Conus*) feed on sedentary and errant polychaetes, although feeding on other worms (sipunculans and nemerteans) and even molluscs has been recorded (Miller, 1989, 1990). This information is derived mainly from gut content analysis.

Radular anatomy of the Conoidea is highly variable both in terms of the number of teeth in a transverse row and in the shape of the teeth. For a long time, radula morphology together with shell characters constituted the basis of the higher classification of the group (e.g., Thiele, 1929; Powell, 1942, 1966; McLean, 1971).

Since about 1990, anatomical investigations of conoideans have revealed great variability in foregut anatomy, and characters defined in these studies have been used to unravel phylogenetic relationships (Taylor, 1990; Taylor et al., 1993; Kantor et al., 1997). Various hypotheses have been proposed concerning the evolutionary transformations in radular morphology of Conoidea (Shimek & Kohn, 1981; Kantor & Taylor, 2000; Kantor, 2006). However, one of the reasons for the lack of a clear understanding of major transformations of the radula is that radula evolution was inferred from phylogenetic hypotheses themselves based partially on radular morphology (e.g., Taylor et al., 1993). As a consequence, many parallel evolutionary transformations cannot be traced.

The rapid development of molecular phylogenetics provided new insight and revolutionary changes in our understanding of conoidean evolution. DNA sequences were first used to infer phylogenetic relationships within genera or subfamilies (e.g., Duda & Palumbi, 1999; Espiritu et al., 2001; Duda & Kohn, 2005; Heralde et al., 2007; Holford et al., 2009) and then among most of the families and subfamilies (as erected by Powell, 1942, 1966; McLean, 1971; Taylor et al., 1993) of the Conoidea (Puillandre et al., 2008). An updated molecular phylogeny based on three mitochondrial genes (COI, 12S and 16S) and including representatives of 102 genera was recently proposed (Puillandre et al., 2011). The single recognized taxon missing from the analysis was the (sub)family Strictispiridae McLean, 1971. Most of the clades inferred have robust support that allowed the status of the different families and subfamilies previously proposed to be clarified and led to a new classification of the group into 15 families (Bouchet et al., 2011).

The molecular framework provides an opportunity to reconstruct the transformation of the morphological characters and to test previously proposed hypotheses. This approach has demonstrated the independent loss of the venom gland in two independent lineages of Terebridae (Holford et al., 2009), but is still not widely used in Conoidea. Here we attempt for the first time a reconstruction of the major morphological transformations of the radular apparatus in Conoidea based on the molecular phylogeny. Understanding the transformations of the radular apparatus is important not only for understanding the evolution of the group in general, but also because it may provide new insight into the factors leading to hyperdiversifi-

cation of the group that led to the appearance of probably the most species-rich marine mollusk taxon. Furthermore, clarifying the evolution of the group, and in particular the evolution of characters linked to the venom apparatus, should be of great value in the discovery of new venom compounds with pharmacological applications (Olivera, 2006; Puillandre & Holford, 2010).

MATERIAL AND METHODS

Radula Preparation

Of the 102 ingroup species in the molecular analysis (Puillandre et al., 2011), the radula of 51 species was examined (in most cases using the same specimens as used for the molecular analysis), ten species were radula-less, and for 13 species published data were used (Table 1). In most cases, unavailability of the radula was explained by destruction of the body during DNA extraction (usually for very small specimens). For six species, radular characters were examined using congeners. The generic position of most of these was confirmed by other molecular data. These species are marked by an asterisk on the molecular trees (e.g., Figs. 5, 6). The complete range of variability of the radula in Conoidea is not, however, covered by the species in our tree (only a single species of each genus was used in the analysis). Some species with important or unique radular morphology are not included in the current analysis, but we inferred their phylogenetic position from separate molecular analyses and therefore mention some of them in the discussion. For the outgroups, radula was examined in conspecific specimens or congeners. Photographs of the radulae are arranged in plates on the taxonomical basis to make the comparison easier.

The radulae were cleaned with diluted bleach (1 part of commercially available bleach to 3–4 parts of distilled water). Cleaning radulae in bleach does not damage radular teeth or the subradular membrane if used in the correct concentration and if the radulae are not exposed to bleach for a long time. Furthermore, soft tissues are diluted in bleach rapidly (usually within a few minutes), allowing continuous observation under the microscope that reveals many important features that otherwise can easily be overlooked, for example, folding of the radular membrane, attachment of radular teeth to the membrane, and presence of a ligament.

The larger radulae were cleaned in a watch glass, while most tiny ones – in a drop of water placed on a cover-slip. For latter ones, the bleach was added either with a syringe or a minute plastic pipette. After dissolving the soft tissues, the radula or separate radular teeth were transferred with a needle or single hair into a drop of clean water on the same cover-slip. This minimizes the chance of losing the small radulae. Two changes of water were usually enough to rinse the radula. After rinsing, the radula was partially pulled out of the drop so that the extruded part adheres to the glass by surface tension. This permits the radula membrane to be more easily unfolded with a single hair, and allows individual teeth to be placed in the desired position prior to drying. The radula was then completely pulled out of the water drop and allowed to dry. The cover-slip was then mounted on the stub. Although simple, this method provides excellent results, allowing manipulating objects smaller than 100 μm in length.

Tree Mapping

Within the Neogastropoda, the sister-group of Conoidea is the rachiglossate group of superfamilies (Oliverio & Modica, 2010). Consequently, we included in our analyses several outgroups from four families of Rachiglossa: Costellariidae (*Vexillum costatum*), Harpidae (*Harpa kajiymai*), Buccinidae (*Belomitra brachytoma*) and Fasciolaridae (*Turriturritus*). Two other distant outgroups were also included: *Xenophora solarioides* (Xenophoridae, Littorinimorpha) and *Laevistrombus guidoi* (Strombidae, Littorinimorpha).

The details of the phylogenetic analysis is provided in Puillandre et al. (2011).

Nine radular morphological characters coded as 31 character states (Table 2) were used to reconstruct the radular transformation. Characters were mapped on the tree of Puillandre et al. (2011) using Mesquite Version 2.74 (Maddison & Maddison, 2007–2010), with the option “tracing character history” and the parsimony ancestral reconstruction method. Most of the characters were treated as unordered. For the characters describing central and lateral teeth (characters 1 and 2 in Table 2), the stepmatrix model was tried in addition to the unordered; it allows interdicting some of the transformation sequences, in our case from absent to present, that is interdicting re-appearance of central and lateral teeth after they had been lost.

The familiar classification accepted here is that of Bouchet et al. (2011). For convenience,

TABLE 1. List of specimens (+ data available, - data unavailable).

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Borsonidae	<i>Bathytoma</i>	<i>neocaledonica</i> Puillandre et al., 2010	MNHN IM200717857	EBISCO, CP2551, 21°060'S, 158°350'E, 637–650 m	+
	<i>Borsonia</i>	sp.	MNHN IM200717932	Salomon 2, CP2197 8°24.40'S, 159°22.50'E, 897–1,057 m	+
	Borsoniidae gen. 1	sp.	MNHN IM200717911	Panglao 2005, CP2333, 9°38.20'N, 123°43.50'E, 584–596 m	+
	<i>Genota</i>	<i>mitriformis</i> (W. Wood, 1828)	MNHN IM200742293	Angola, AF7, Pta. Das Lagostas	+
	<i>Microdrillia</i>	cf. <i>optima</i> (Thiele, 1925)	MNHN IM200717887	Panglao 2004, T36, 9°29.30'N, 123°51.50'E, 95–128 m	-
	<i>Tomopleura</i>	<i>reevii</i> (C. B. Adams, 1850)	MNHN IM200717875	Panglao 2004, T26, 9°43.30'N, 123°48.80'E, 123–135 m	-
	<i>Typhlomangelia</i> (cf.)	sp.	MNHN IM200717931	Salomon 2, CP2269, 7°45.10'S, 156°56.30'E, 768–890 m	-
	<i>Zemacies</i>	<i>excelsa</i> Sysoev & Bouchet, 2001	MNHN IM200911056	Musorstom 4, DW226, 22°47'S, 167°22'E, 395 m	Radula-less species
	<i>Clathurella</i>	<i>nigrotincta</i> (Montrouzier, 1872)	MNHN IM200742607	Santo 2006, VM53, 15°31'S, 167°09'E, intertidal	+
	<i>Etrema</i>	cf. <i>tenera</i> (Hedley, 1899)	MNHN IM200717869	Panglao 2004, S21, 9°41.70'N, 123°50.90'E, 4–12 m	-
Clavatulidae	<i>Nannodiella</i>	<i>ravella</i> (Hedley, 1922)	MNHN IM200717904	Panglao 2004, T9, 9°33.5'N, 123°49.50'E, 97–120 m	+
	<i>Clavatula</i>	<i>xanteni</i> Nolf & Verstraeten, 2006	MNHN IM200717829	Angola, AF1, 8°780'S, 13°230'E, 40–60 m	+
	<i>Gemmuloborsonia</i>	<i>colorata</i> (Sysoev & Bouchet, 2001)	MNHN IM200717849	EBISCO, DW2619, 20°060'S, 160°230'E, 490–550 m	+
	<i>Perrona</i>	<i>subspirata</i> (Martens, 1902)	MNHN IM200717833	Angola, AF10, 15°140'S, 12°290'E, 50 m	+
	<i>Pusionella</i>	<i>compacta</i> Strebel, 1914	MNHN IM200717830	Angola, AF3, 10°510'S, 14°230'E, 5–10 m	+

(continues)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Cochlespiridae	<i>Turricula</i>	<i>nelliae</i> <i>spurius</i> (Hedley, 1922)	NHМУK MOEA 20100552	Off southern Hong Kong, South China Sea	+
	<i>Cochlespira</i>	<i>pulchella</i> (Schepman, 1913)	MNHN IM200717920	Panglao 2005, CP2340, 9°29.40'N, 123°44.40'E, 271–318 m	+
	<i>Sibogasyrinx</i>	sp.	MNHN IM200717701	BOA1, CP2432, 14°59.70'S, 166°55.00'E, 630–705 m	+
Conidae	<i>Californiconus</i>	<i>californicus</i> (Hinds, 1844)		Monterey, California	Tucker & Tenorio, 2009
	<i>Conasprella</i>	<i>pagoda</i> (Kiener, 1845)	MNHN IM200717914	Panglao 2005, CP2380, 8°41.30'N, 123°17.80'E, 150–163 m	Tucker & Tenorio, 2009
	<i>Conus</i>	<i>consors</i> G. B. Sowerby I, 1833	MNHN IM200717939	Santo 2006, AT87, 15°32.10'S, 167°16.10'E, 235–271 m	Tucker & Tenorio, 2009
Conorbidae	<i>Profundiconus</i>	<i>teramachii</i> (Kuroda, 1956)		Philippines	Tucker & Tenorio, 2009
	<i>Taranteconus</i>	<i>chiangi</i> Azuma, 1972		Philippines	Tucker & Tenorio, 2009
	<i>Benthofascis</i>	<i>lozoueti</i> Sysoev & Bouchet, 2001	MNHN IM200742331	Norfolk 2, DW2147, 22°50'S, 167°16'E, 496 m	+
Drilliidae	<i>Agladrillia</i>	<i>pudica</i> (Hinds, 1843)	NHМУK MOEA 20100543	Gulf of Panama, JTD-00-51, 8°36.41'N, 79°09.70'W, 73 m	McLean, 1971
	<i>Calliclava</i>	sp.	NHМУK MOEA 20100546	Gulf of Panama, JTD-00-47, 8°31.83'N, 79°05.09'W, 21 m	McLean, 1971
	<i>Cerodrillia</i>	<i>cybele</i> (Pilsbry & Lowe, 1932)	NHМУK MOEA 20100548	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	McLean, 1971
	<i>Clathrodrillia</i>	<i>walteri</i> (M. Smith, 1946)	NHМУK MOEA 20100549	Gulf of Panama, JTD-00-46, 8°31.37'N, 79°05.79'W, 24–25 m	-
	<i>Clavus</i>	<i>canalicularis</i> (Roeding, 1798)	MNHN IM200717858	Panglao 2004, S12, 9°29.40'N, 123°56.00'E, 6–8 m	+
	<i>Conopleura</i>	<i>striata</i> Hinds, 1844	MNHN IM200717889	Panglao 2004, T41, 9°29.70'N, 123°50.20'E, 110–112 m	-
	<i>Cruziturracula</i>	<i>arcuata</i> (Reeve, 1843)	NHМУK MOEA 20100541	Gulf of Panama, JTD-00-34, 8°26.24'N, 79°09.14'W, 66–68 m	+

(continued)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Horaiclavidae	<i>Drillia</i>	<i>clavata</i> (G. B. Sowerby III, 1870)	NHMUK MOEA 20100550	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	McLean, 1971
	<i>Fusiturricula</i>	<i>enae</i> (Bartsch, 1934)	INVEMAR MOL-1929	Colombia, E-73, 9°57.53'N, 79°07.71'W, 268–270 m	-
	<i>Imaclava</i>	<i>unimaculata</i> (Sowerby I, 1834)	NHMUK MOEA 20100527	Gulf of Panama, JTD-00-46, 8°31.37'N, 79°05.79'W, 24–25 m	Shimek & Kohn, 1981
	<i>Iredalea</i>	<i>pupoidea</i> (H. Adams, 1872)	MNHN IM200742556	Santo 2006, DB25, 15°37.7'S, 167°11.3'E, 10 m	+
	<i>Splendrillia</i>	sp.	MNHN IM200717847	EBISCO, DW2617, 20°060'S, 160°220'E, 427–505 m	+
	<i>Anacithara</i>	<i>lita</i> (Mellvill & Standen, 1896)	MNHN IM200742614	Santo 2006, DS99, 15°32'S, 167°17'E, 100–105 m	+
	<i>Anguloclavus</i>	sp.	MNHN IM200717908	Panglao 2005, CP2332, 9°38.80'N, 123°45.90'E, 396–418 m	+
	<i>Carinapex</i>	<i>minutissima</i> (Garrett, 1873)	MNHN IM200717868	Panglao 2004, B19, 9°29.40'N, 123°56.00'E, 17 m	+
	<i>Ceritoturris</i>	<i>pupiformis</i> (Smith, 1884)	MNHN IM200717888	Panglao 2004, T36, 9°29.30'N, 123°51.50'E, 95–128 m	+
	<i>Horaiclavidae</i> gen. 1	sp. (juvenile)	MNHN IM200742501	Salomon 2, CP2219, 7°58'S, 157°34'E, 650–836 m	+
	<i>Horaiclavus</i>	<i>splendidus</i> (A. Adams, 1867)	MNHN IM200717840	EBISCO, DW2631, 21°030'S, 160°440'E, 372–404 m	+
	<i>Paradrillia</i>	sp. (juvenile)	MNHN IM200742475	Panglao 2005, CP2396, 9°36'N, 123°42'E, 609–673 m	+
MangelIIDae	<i>Anticlinura</i>	sp.	MNHN IM200742513	Salomon 2, CP2182, 8°47'S, 159°38'E, 762–1060 m	+
	<i>Benthomangelia</i>	cf. <i>trophonoidea</i> (Schepman, 1913)	MNHN IM200717835	BOA1, CP2462, 16°37.50'S, 167°57.40'E, 618–641 m	+
	<i>Eucithara</i>	cf. <i>coronata</i> (Hinds, 1843)	MNHN IM200717900	Panglao 2004, B8, 9°37.10'N, 123°46.10'E, 3 m	+

(continues)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Mitromorphidae	<i>Heterocithara</i>	sp.	MNHN IM200717884	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110 m	-
	Mangeliidae gen. 1	sp.	MNHN IM200717874	Panglao 2004, T26, 9°43.30'N, 123°48.80'E, 123–135 m	Radula of <i>Mangelia powisiana</i> (Dautzenberg, 1887) used
	Mangeliidae gen. 2	sp.	MNHN IM200717872	Panglao 2004, S26, 9°41.50'N, 123°51.00'E, 21 m	-
	<i>Oenopota</i>	sp.	MNHN IM200742325	Hornsund, Svalbard, 1184–2001	-
	<i>Toxicochlespira</i>	<i>pagoda</i> Sysoev & Kantor, 1990	MNHN IM200717925	Salomon 2, CP2227, 6°37.20'S, 156°12.70'E, 508–522 m	+
	<i>Lovellona</i>	<i>atramentosa</i> (Reeve, 1849)	MNHN IM200742552	Santo 2006, NR8, 15°35.7'S, 167°07.4'E, 11 m	+
	<i>Mitromorpha</i>	<i>metula</i> (Hinds, 1843)	MNHN IM200717898	Panglao 2004, B8, 9°37.10'N, 123°46.10'E, 3 m	+
	<i>Carinodrillia</i>	<i>dichroa</i> Pilsbry & Lowe, 1932	NHMUK MOEA 20100530	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	+
	<i>Cheungbeia</i>	<i>robusta</i> (Hinds, 1839)	NHMUK MOEA 20100556	Off southern Hong Kong, South China Sea, Sta. 70	+
	<i>Comitas</i>	sp.	MNHN IM200717918	Panglao 2005, CP2388, 9°26.90'N, 123°34.50'E, 762–786 m	+
Pseudomelatomidae	<i>Crassispira</i>	<i>quadrilirata</i> (E. A. Smith, 1882)	MNHN IM200717755	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110 m	+
	<i>Funa</i>	<i>incerta</i> (Smith, 1877)	NHMUK MOEA 20100533	Off southern Hong Kong, South China Sea, Sta. 70	+
	<i>Hindsiclavava</i>	<i>alesidota</i> (Dall, 1889)	NHMUK MOEA 20100525	Lower Florida Keys, JTD-01-15, 24°33.47'N, 81°07.72'W, 117–148 m	+
	<i>Inquisitor</i>	sp.	MNHN IM200717851	EBISCO, DW2625, 20°050'S, 160°190'E, 627–741 m	+
	<i>Knefastia</i>	<i>tuberculifera</i> (Broderip & Sowerby, 1829)	NHMUK MOEA 20100533	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	+

(continued)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
	<i>Leucosyrinx</i>	sp.	MNHN IM200717846	EBISCO, CP2600, 19°380'S, 158°460'E, 603–630 m	+
	<i>Otitoma</i>	sp.	MNHN IM200717905	Panglao 2005, CP2348, 9°29.60'N, 123°52.50'E, 196–216 m	+
	<i>Pilsbryspira</i>	<i>jayana</i> (C. B. Adams, 1850)	USNM 857830	Carrie Bow Cay, Belize, intertidal	+
	<i>Pseudomelatoma</i>	<i>moesta</i> (Carpenter, 1865)		California	Kantor, 1988
	<i>Ptychobela</i>	<i>suturalis</i> (Gray, 1838)	NHМУK MOEA 20100560	Luong SoN, 15 km N. Nha Trang, Vietnam	+
	<i>Pyrgospira</i>	<i>aenone</i> (Dall, 1919)	NHМУK MOEA 20100539	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	+
	<i>Tiariturreis</i>	<i>spectabilis</i> Berry, 1958	NHМУK MOEA 20100540	Gulf of Panama, JTD-00-34, 8°26.24'N, 79°09.14'W, 66–68 m	+
	<i>Zonulispira</i>	sp.	NHМУK MOEA 20100536	Gulf of Panama, JTD-00-18, 8°19.50'N, 78°47.71'W, 25–32 m	+
	<i>Daphnella</i>	sp.	MNHN IM200717927	Salomon 2, CP2260, 8°03.50'S, 156°54.50'E, 399–427 m	Radula of <i>Daphnella mitrellaformis</i> (Nomura, 1940) used
	<i>Eucyclotoma</i>	<i>cymatodes</i> (Hervier, 1899)	MNHN IM200717903	Panglao 2004, S12, 9°29.40'N, 123°56.00'E, 6–8 m	-
	<i>Glyphostomoides</i> (cf.)	sp.	MNHN IM200717892	Panglao 2004, T39, 9°30.10'N, 123°50.40'E, 100–138 m	-
	<i>Gymnobela</i>	sp.	MNHN IM200717841	EBISCO, CP2648, 21°320'S, 162°300'E, 750–458 m	Radula of <i>Gymnobela yoshidai</i> (Kuroda & Habe in Habe, 1962) used
	<i>Hemilienardia</i>	<i>calcicincta</i> (Melvill & Standen, 1895)	MNHN IM200717861	Panglao 2004, B14, 9°38.50'N, 123°49.20'E, 2–4 m	-
	<i>Kermia</i>	<i>aureotincta</i> (Hervier, 1897)	MNHN IM200717878	Panglao 2004, B25, 9°29.40'N, 123°56.10'E, 16 m	Radula of <i>Kermia irretita</i> (Hedley, 1899) used
	<i>Pleurotomella</i>	sp.	MNHN IM200717848	EBISCO, DW2625, 20°050'S, 160°190'E, 627–741 m	-
	<i>Raphitoma</i>	<i>rubroapicata</i> (E. A. Smith, 1885)	MNHN IM200717890	Panglao 2004, L49, 9°36.50'N, 123°45.30'E, 90 m	Radula of <i>Raphitoma</i> sp. used

(continues)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Terebridae	<i>Rimosodaphnella</i>	sp.	MNHN IM200717836	BOA1, CP2462, 16°37.50'S, 167°57.40'E, 618–641 m	-
	<i>Taranis</i>	sp.	MNHN IM200742296	Aurora 07, CP2749, 15°57'N, 121°50'E, 743 m	Radula-less species
	<i>Teretiopsis</i>	cf. <i>hyalina</i> Sysoev & Bouchet, 2001	MNHN IM200717845	EBISCO, CP2651, 21°290'S, 162°360'E, 883–957 m	Radula-less species
	<i>Thatcheria</i>	<i>mirabilis</i> (Angas, 1877)	MNHN IM200717924	Salomon 2, CP2184, 8°16.90'S, 159°59.70'E, 464–523 m	Kantor & Taylor, 2002
	<i>Tritonoturris</i> (cf.)	sp.	MNHN IM200717891	Panglao 2004, T39, 9°30.10'N, 123°50.40'E, 100–138 m	-
	<i>Veprecula</i>	sp.	MNHN IM200717883	Panglao 2004, L46, 9°30.90'N, 123°41.20'E, 90–110 m	Radula of <i>Veprecula veprectica</i> (Hedley, 1903) used
	<i>Cinguloterebra</i>	<i>cingulifera</i> (Lamarck, 1822)	MNHN IM200716735	Panglao 2005, CP2395, 9°36.2'N, 123°43.8'E, 382–434 m	+
	<i>Clathroterebra</i>	<i>fortunei</i> (Deshayes, 1857)	MNHN IM200730401	Panglao 2005, CP2331, 9°39.2'N, 123°47.5'E, 255–268 m	Radula-less species
	<i>Euterebra</i>	<i>tristis</i> (Deshayes, 1859)	MNZ	New-Zealand	Rudman, 1969
	<i>Hastula</i>	<i>strigilata</i> (Linnaeus, 1758)		Santo 2006, VM24, 15°35.2'S, 167°59.4'E, intertidal	-
	<i>Hastulopsis</i>	<i>pertusa</i> (Born, 1778)	MNHN IM200730480	Santo 2006, FR10, 15°36.9'S, 167°10.5'E, 6–33 m	Radula-less species
	<i>Myurella</i>	<i>kilburni</i> (Burch, 1965)	MNHN IM200730459	Panglao 2004, S18, 9°35.7'N, 123°44.4'E, 0–2 m	Radula-less species
	<i>Oxymoris</i>	<i>areolata</i> (Link, 1807)	MNHN IM200730371	Santo 2006, NR5, 15°28.7'S, 167°15.2'E, 19 m	Radula-less species
	<i>Strioterebrum</i>	<i>plumbum</i> (Quoy & Gaimard, 1833)	MNHN IM200730558	Santo 2006, ED5, 15°31'S, 167°09'E, intertidal	Radula-less species
	<i>Terebra</i>	<i>textilis</i> Hinds, 1844	MNHN IM200717938	Santo 2006, LD28, 15°35.40'S, 166°58.70'E, 3–8 m	Radula-less species
	<i>Terenolla</i>	<i>pygmaea</i> (Hinds, 1844)	MNHN IM200730449	Panglao 2004, S2, 9°37.4'N, 123°54.5'E, 4–5 m	Radula-less species

(continues)

(continued)

Family	Genus	Species	Institutional registration number	Expedition/locality, station and depth	Radula preparation or source of information
Turridae	<i>Gemmula</i>	<i>rarimaculata</i> Kuroda & Oyama, 1971	MNHN IM200717838	EBISCO, DW2533, 22°180'S, 159°280'E, 360–370 m	+
	<i>Iotyrriis</i>	<i>cingulifera</i> (Lamarck, 1822)	MNHN IM200717685	Santo 2006, FS84, 15°33.6'S, 167°16.6'E, 8–9 m	+
	<i>Lophiotoma</i>	<i>acuta</i> (Perry, 1811)	MNHN IM200717860	Panglao 2004, R44, 9°33.30'N, 123°43.90'E, 2 m	+
	<i>Lucerapex</i>	<i>cf. casearia</i> (Hedley & Petterd, 1906)	MNHN IM200742448	Panglao 2005, CP2363, 9°06'N, 123°25'E, 437–439 m	+
	<i>Polystira</i>	<i>albida</i> (Perry, 1811)	NHMUK MOEA 20110066	S. of Bahia Honda Key, Florida Keys, 24834.24'N; 81816.64'W, 30–34 m	Bandel, 1984
	<i>Ptychosyrinx</i>	<i>carynae</i> (Haas, 1949)	USNM 832922	North Atlantic, 38°14'N, 70°29.28'W, 3,188–5,300 m	-
	<i>Turridrupa</i>	<i>cf. armillata</i> (Reeve, 1845)	MNHN IM200717850	EBISCO, DW2607, 19°330'S, 158°400'E, 400–413 m	-
	<i>Turris</i>	<i>babylonia</i> Linnaeus, 1758	MNHN IM200717754	Panglao 2004, R42, 9°37.10'N, 123°52.60'E, 8–22 m	A. E. Fedosov, unpublished
	<i>Xenuroturris</i>	<i>legitima</i> Iredale, 1929	MNHN IM200717684	Santo 2006, DR87, 15°38.5'S, 167°15.1'E, 13 m	+
	<i>Belomitra</i>	<i>brachytoma</i> (Schepman, 1913)	MNHN IM200911057	Salomon 2, CP2184, 8°17'S, 160°00'E, 464–523 m	+
Outgroups	<i>Harpa</i>	<i>kajiyamai</i> Habe & Kosuge, 1970	MNHN IM200740569	Santo 2006, EP22, 15°37.3'S, 167°05.8'E, 78–91 m	Hughes & Emerson, 1987
	<i>Laevistrombus</i>	<i>guidoi</i> (Man in't Veld & De Turck, 1998)	MNHN IM200911060	Santo 2006, LR3, 15°35.8'S, 167°06.1'E	Bandel, 1984
	<i>Turrilatirus</i>	<i>turritus</i> (Gmelin, 1791)	MNHN IM200911059	Santo 2006, FB52, 15°42.7'S, 167°15.1'E, 7 m	Radula of <i>Latirus</i> sp. used
	<i>Vexillum</i>	<i>costatum</i> (Gmelin, 1791)	MNHN IM200911058	Santo 2006, DR64, 15°27.6'S, 167°14.3'E, 6–35 m	+
	<i>Xenophora</i>	<i>solarioides</i> (Reeve, 1845)	MNHN IM200911061	Santo 2006, AT55, 15°36.2'S, 167°02.5'E, 80–82 m	Bandel, 1984

TABLE 2. List of characters states used in the analysis.

No.	Charater description	Character states				
		0	1	2	3	4
1	morphology of central tooth	absent	multicuspid of caenogastro-pod type	multicuspid of neogastropod type	unicuspid narrow	unicuspid broad
2	morphology of the lateral teeth	absent	cuspidate of caenogastro-pod type	cuspidate of neogastropod type	unicuspid of neogastro-pod type	plate-like
3	morphology of the marginal teeth	absent	non-duplex (flat)	duplex or semi-enrolled	solid, recurved	hypodermic
4	duplex marginal teeth	marginal teeth absent	teeth with un-equal limbs	teeth with sub-equal limbs	teeth of <i>Comitas</i> type	semi-enrolled
5	details of hypodermic marginal teeth - spur	absent	present	hypodermic teeth absent		
6	details of hypodermic marginal teeth – barbs	absent	one barb present	two barbs present	three or more barbs present	hypodermic teeth absent
7	details of hypoder-mic marginal teeth - blade	absent	present	hypodermic teeth absent		
8	details of hypodermic marginal teeth – liga-ment	absent	present	hypodermic teeth absent		
9	use of marginal teeth at proboscis tip	separate tooth used at the proboscis tip	separate tooth not used at the proboscis tip	marginal teeth absent		

in addition to the families recognized by Bouchet et al. (2011), we refer to major clades A and B (without attributing a taxonomic status to them) that are different in many aspects of anatomy and radular morphology.

Acronyms for Depositories of Voucher Specimens

INVEMAR	Instituto de Investigaciones Marinas y Costeros, Santa Marta, Colombia
MNHN	Muséum National d’Histoire Naturelle, Paris, France
MNZ	Museum of New Zealand Te Papa Tongareva, Wellington, New Zealand
NHMUK	The Natural History Museum, London, U.K.
USNM	National Museum of Natural History, Smithsonian Institution, Washington D.C., U.S.A.

RESULTS

Although the radulae of Conoidea have been described in many publications, the thorough use of scanning electron microscopy revealed many previously overlooked characters and allowed new interpretations of structures already described. Therefore, we provide here a much updated overview of the radular characters. The evolution of each of the nine characters analysed is described.

Character 1: Central Tooth of the Radula (Figs. 5, 6)

A central tooth is present in all outgroups. It is absent in clade A and present in some groups of clade B (Figs. 7–41). The morphology of the central tooth is rather variable in gastropods. Two major types are found in the outgroups, both multicuspid. Describing in detail the

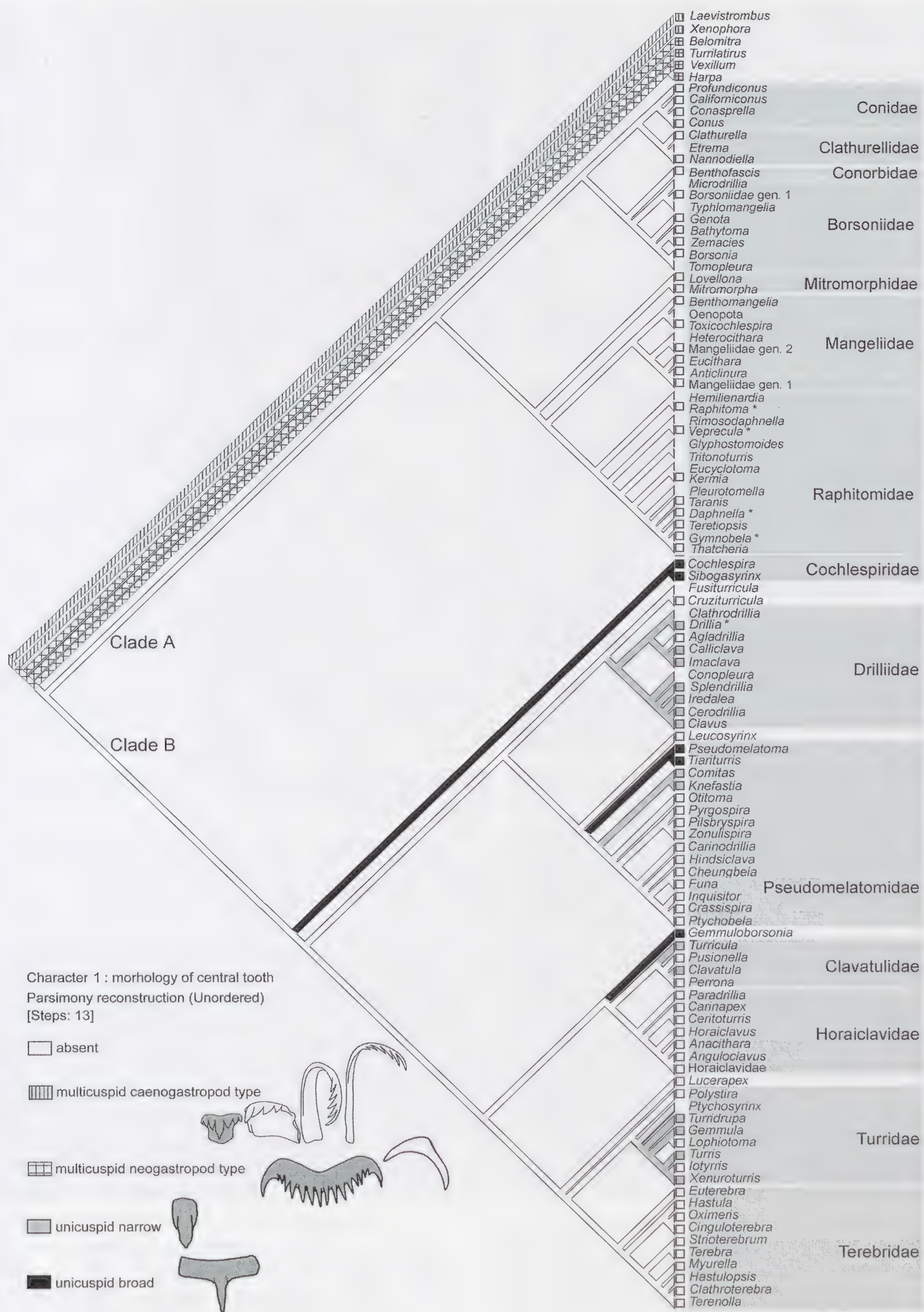


FIG. 5. Evolution of the central radular tooth morphology (character 1) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. The different shading and tiling of the branches corresponds to the most parsimonious ancestral state for the corresponding clade. The mixed shading and/or tiling of the branch indicates that analysis was not able to resolve the single most parsimonious state.

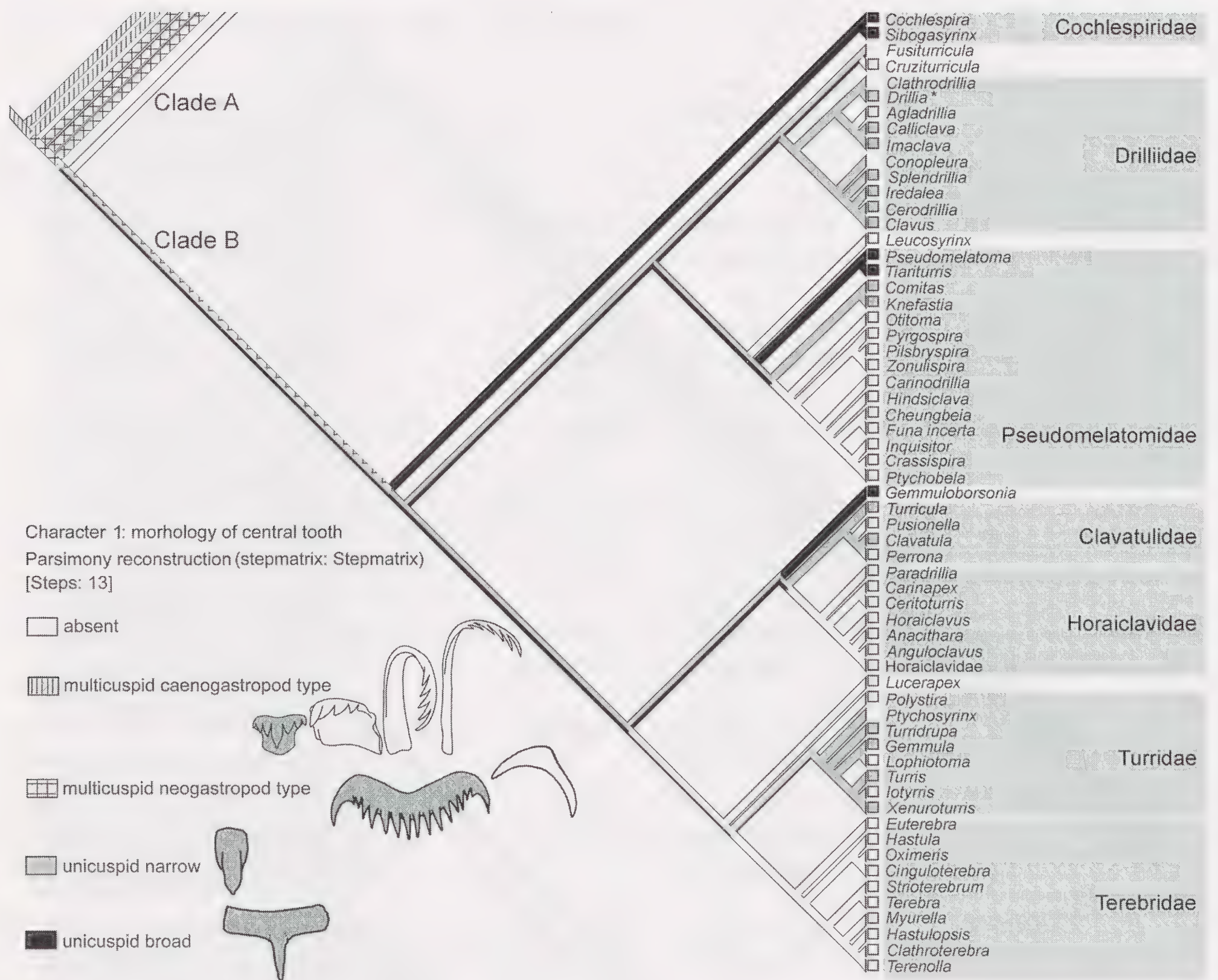


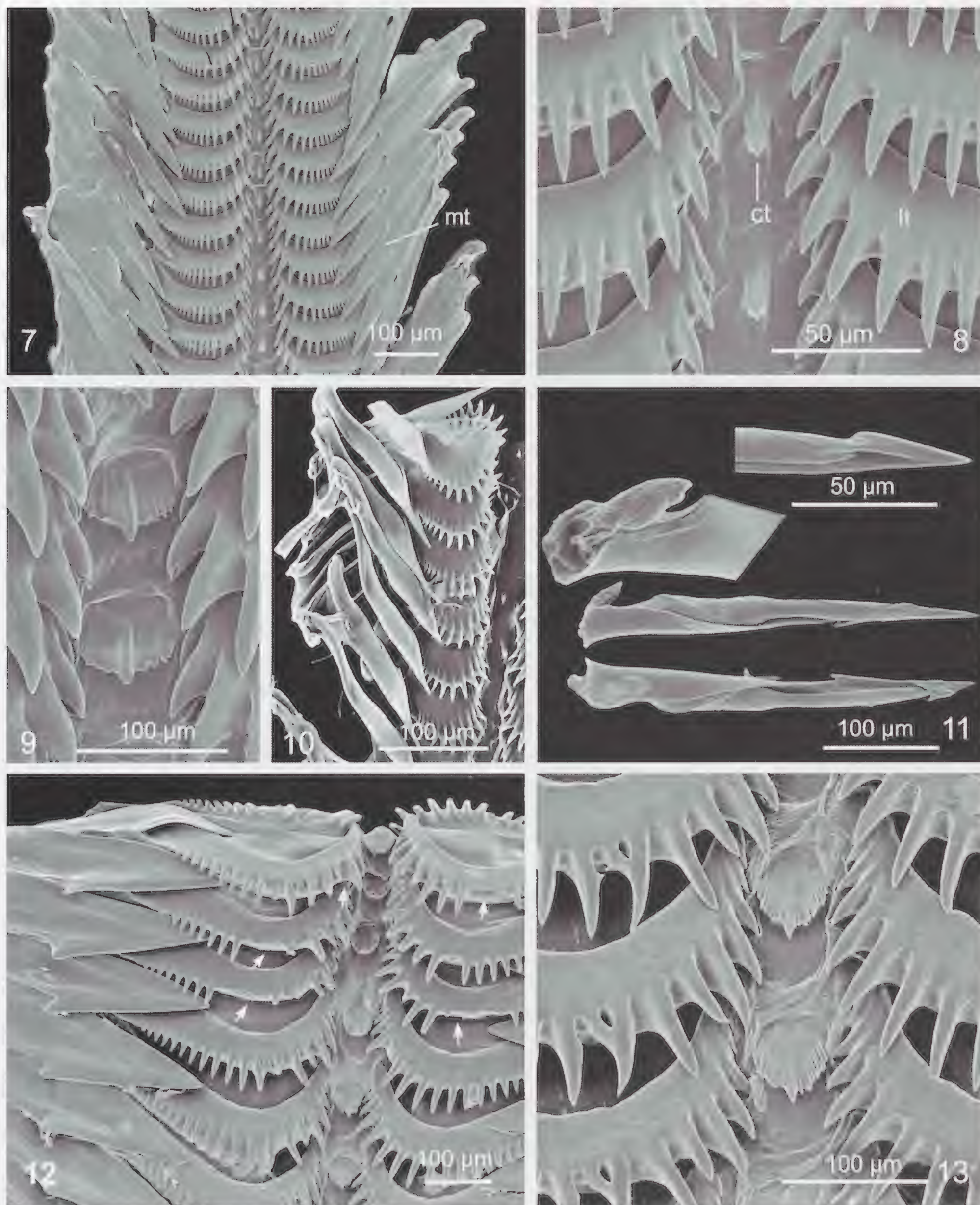
FIG. 6. Evolution of the central radular tooth morphology mapped on the conoidean molecular phylogeny (character 1). A stepmatrix parsimony model was used, interdicting reversion of the character states. Since central teeth are absent in the entire clade A, only clade B is illustrated.

morphology of the teeth of groups other than Conoidea (e.g., Bandel, 1984) is outside the scope of the current paper. In Conoidea with a central tooth, its form varies widely. Two major types of well-defined central teeth can be identified: narrow unicuspid (shield-like with a small cusp and sometimes with additional serrations) (Figs. 7–9, 12, 13); and broad unicuspid, with a large curved cusp and well-defined lateral flaps (Fig. 14).

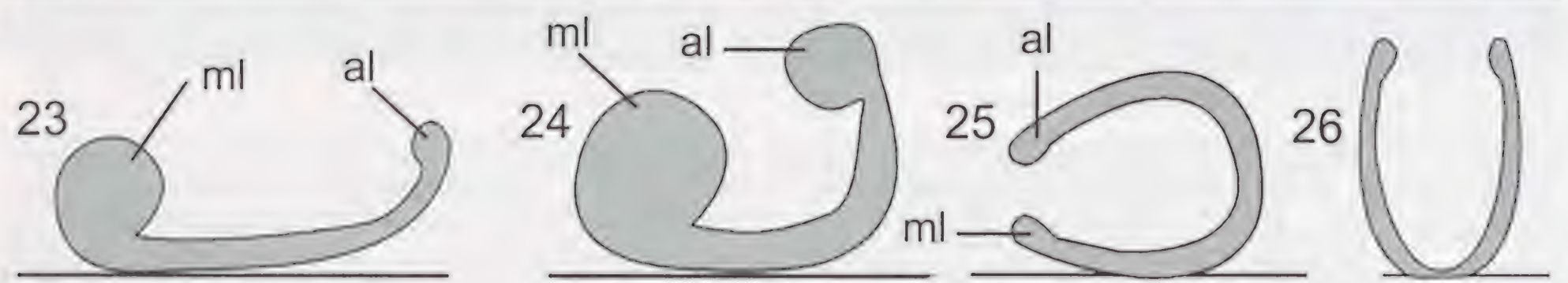
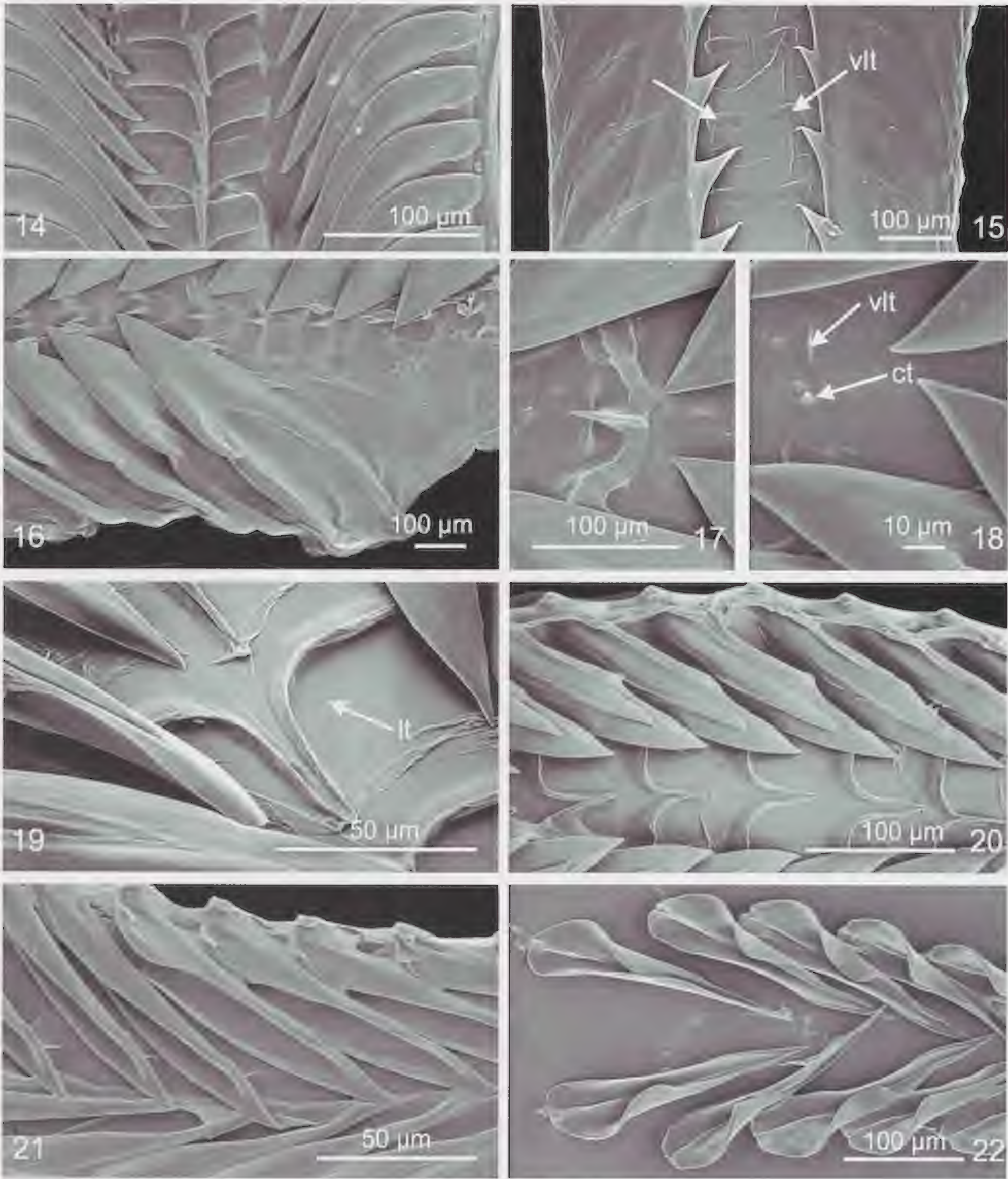
Conversely, in a number of Conoidea from clade B, vestigial rather indistinct structure(s) occupy the middle portion of the subradular membrane (Figs. 28, 30 – marked with arrows). In some cases, they can be hardly seen without staining the radular membrane or observing under SEM. These structures may be either a much reduced broad central tooth with lateral flaps, with or without a central cusp, or alterna-

tively three teeth – vestigial central and vestigial laterals (see below) partially or completely fused (Figs. 17, 18). Vestigial structures were found in some Pseudomelatomidae, Clavatulidae and Turridae (*Iotyrrix* Medinskaya & Sysoev, 2001).

Narrow unicuspid central teeth are found in most Drilliidae and some Turridae (in our dataset exemplified by species of *Xenuroturrus* Iredale, 1929, and *Turridrupa* Hedley, 1922 – Figs. 33, 34, as well as *Gemmula* and *Turris*). A broad, well-defined central tooth was recorded in three clades – in some Pseudomelatomidae (*Pseudomelatoma*, *Hormospira* Berry, 1958, and *Tiariturrus* Berry, 1958 – Fig. 14), Cochlespiridae (Fig. 39), and *Gemmuloborsonia* Shuto, 1989 (not currently attributed to any family – Fig. 32). In these genera, the posterior edge of the tooth, bearing the cusp, is well elevated over the membrane.



FIGS. 7–13. Radulae of Drilliidae. If not otherwise mentioned, data for the specimens are given in Table 2. FIGS. 7, 8: *Splendrillia* sp., MNHN IM200717847; FIG. 9: *Clavus exasperatus* (Reeve, 1843), MNHN, New-Caledonia, LIFOU 2000, st. 1420, 20°47.7'S, 167°09.35'E, 4–5m; FIG. 10: *Imaclava pilsbryi* (Bartsch, 1950), after Kantor & Taylor, 2000; FIG. 11: *Cruziturricula arcuata* (Reeve, 1843), NHMUK MOEA 20100541. Semi-enrolled marginal teeth; FIGS. 12, 13: *Clavus* sp. 3, MNHN uncataloged, BATHUS 2, DW714; FIG. 12: Bending plane of the radula, arrows indicate strong wear of the teeth; FIG. 13: Central part of the same radula, showing intact central and lateral teeth. Abbreviations: ct – central tooth; lt – lateral tooth, mt – marginal tooth.



Four analyses were performed. In the first, the inconspicuous central teeth, when recognized, were considered as unicuspid narrow (lateral flaps were considered as vestigial plate-like lateral teeth), and character states were unordered (Fig. 5). The tree was 13 steps long and suggested that the plesiomorphic condition of the character in clade B is the absence of the central tooth and the central teeth originated independently in eight clades. Since central teeth are present in the outgroups, this can be considered a reversion. However, the presence of numerous reversions within clade B seems rather unlikely.

Therefore, the second analysis was performed with reversions interdicted (stepmatrix parsimony model) (Fig. 6). The tree was 18 steps long (five steps longer), and the analysis did not allow reconstruction of the single most parsimonious state in Clade B, since a multicuspid tooth (characteristic for Neogastropoda), a narrow unicuspid tooth and a broad unicuspid tooth are equally parsimonious. This analysis suggested independent losses of central teeth in several clades: most species of Pseudomelatomidae, *Leucosyrinx* Dall 1889, Horaclavidae and Terebridae, as well as in some species of Turridae and Clavatulidae.

The third and fourth analyses were with alternative coding of the characters and with unordered and stepmatrix parsimony models correspondingly. Species with vestigial central structures were coded as having the broad unicuspid teeth. The reconstruction produced longer trees (15 and 20 steps, respectively), which were therefore rejected.

Character 2: Lateral Teeth of the Radula (Fig. 42)

Lateral teeth are present in all outgroups. They are absent in Clade A and present in some groups of Clade B.

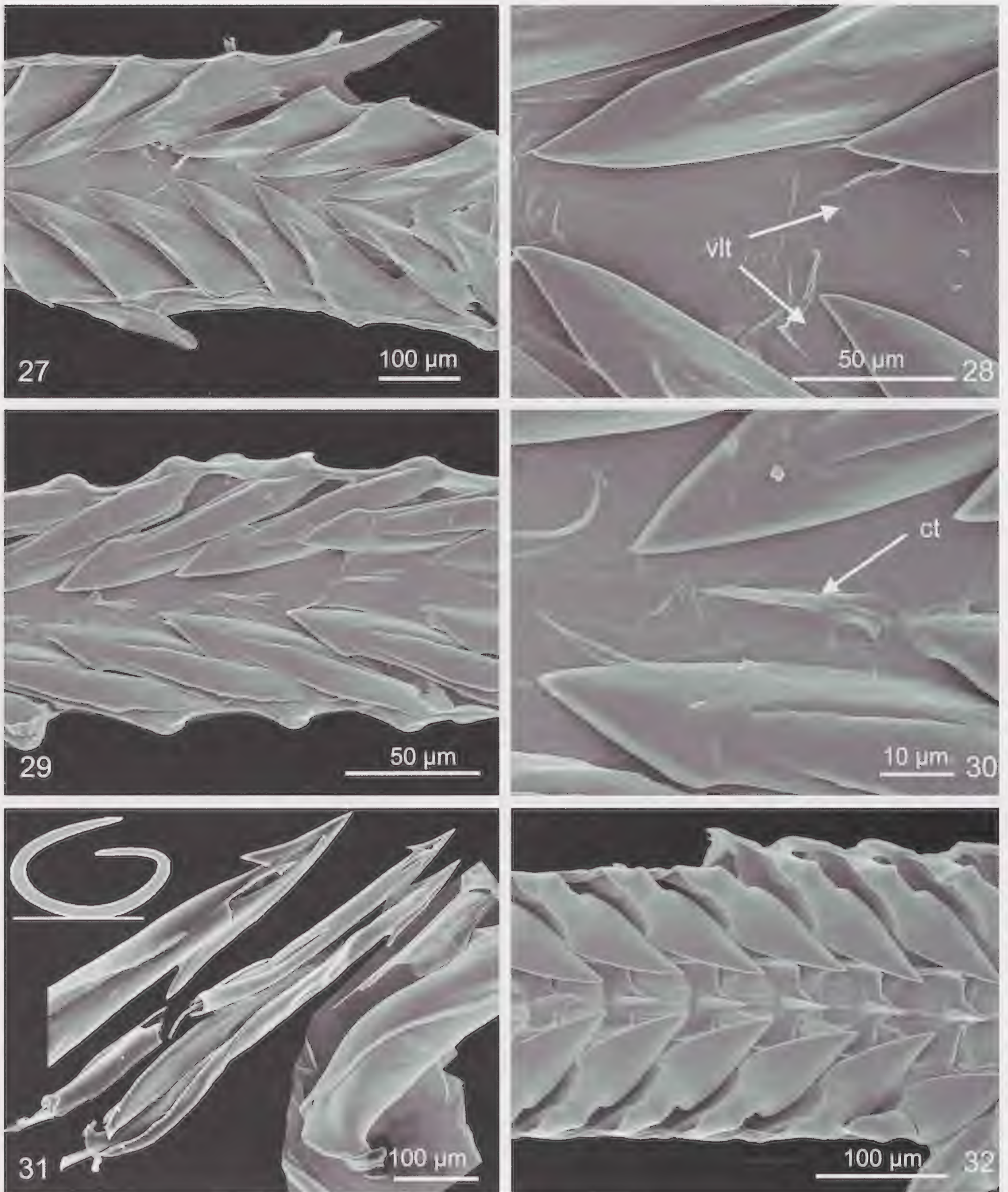
There are two major types of lateral teeth among the ingroup species in our tree. In Drilliidae, they are well formed and multicuspid, completely separate from the central tooth (Figs. 7–10). In all others (some Pseudomelatomidae, Turridae and Clavatulidae), they are very weak, plate-like, non-cuspidate and usually completely or partially fused with the central tooth (when it is present), forming the “central formation” (Kantor, 2006) (see discussion below). In some groups, the laterals are so weak that their presence can be revealed only by staining of the subradular membrane. This is particularly characteristic in Clavatulidae, in which they were first revealed by Kilburn (1985).

A first analysis with character states unordered suggested the absence of lateral teeth is ancestral for the Conoidea and independent appearance of the lateral teeth occurred independently in five clades (all in clade B). Since lateral teeth are present in outgroups, these events would be considered as a reversions.

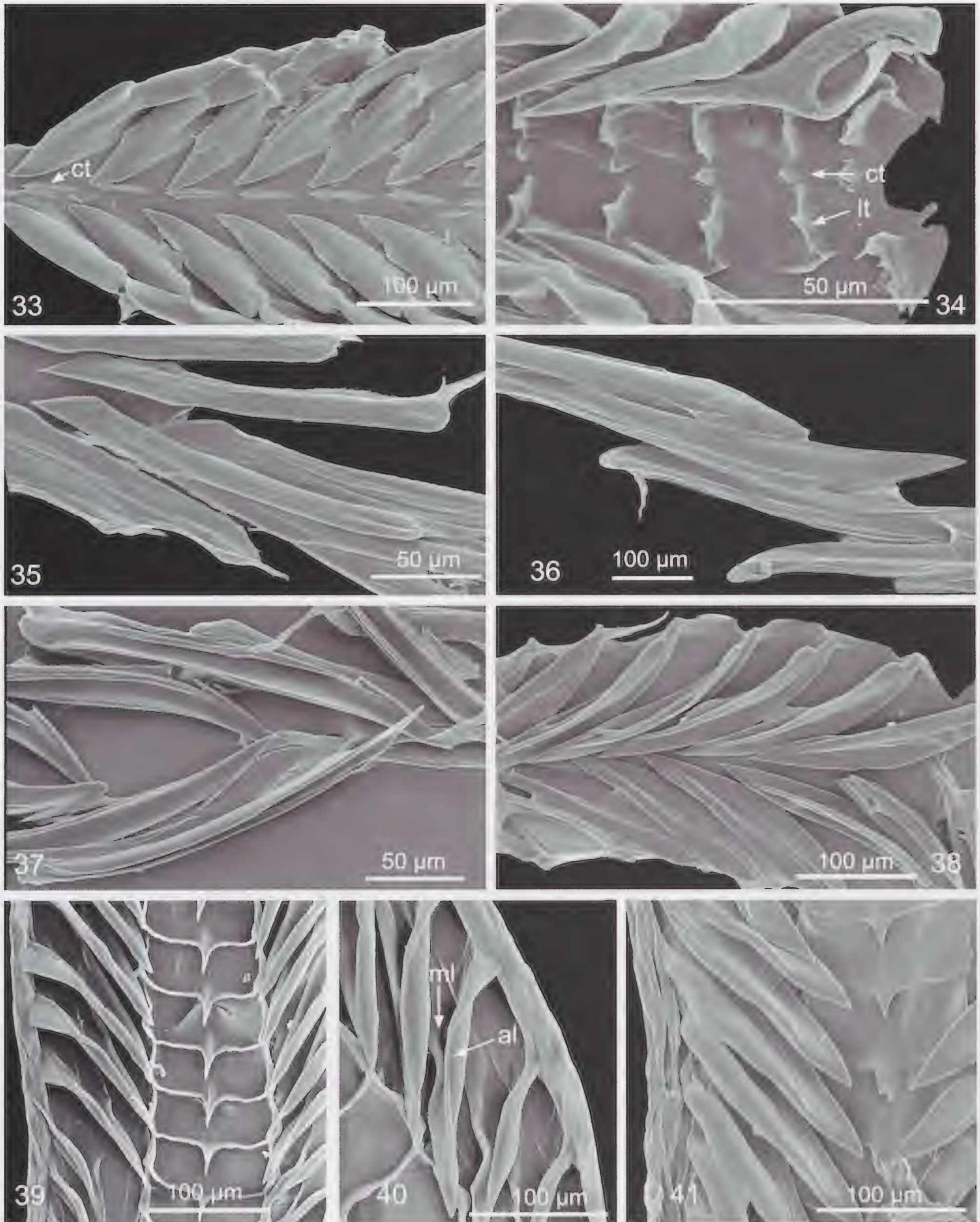
The second analysis was performed with reversions interdicted (Fig. 42) and resulted in a longer tree (17 steps vs. nine in the previous analysis). The analysis did not allow reconstruction of the single most parsimonious state for the entire Conoidea nor for Clade B, since multicuspid neogastropod type teeth, unicuspid

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FIGS. 14–26. Radulae of Pseudomelatomidae. If not otherwise mentioned, data for the specimens are given in Table 2. FIG. 14: *Tiariturris spectabilis* Berry, 1958, NHMUK MOEA 20100540. Radula with solid recurved marginal teeth and broad central teeth; FIG. 15: *Comitas onokeana vivens* Dell, 1956, MNHN, New-Caledonia, MONTROUZIER, st. 1269, after Kantor & Taylor, 2000. Radula with paired plate-like lateral teeth; FIGS. 16, 17: *Comitas* sp., MNHN IM200717918; FIG. 17: Enlarged central segment of the radula; FIG. 18: *Knefastia tuberculifera* (Broderip & Sowerby, 1829), NHMUK MOEA 20100533. Enlarged central segment of the radula; FIGS. 19, 20: *Crassiclava turricula* (Sowerby, 1834). Costa Rica, Off Nacascos, west side of Bahia Culebra, after Kantor et al., 1997. Radula with paired plate-like lateral teeth; FIG. 21: *Zonulispira* sp., NHMUK MOEA 20100536. Radula with semi-enrolled marginal teeth; FIG. 22: *Ptychobela suturalis* (Gray, 1838), NHMUK MOEA 20100560. Radula with semi-enrolled marginal teeth; FIGS. 23–26: Diagrammatic transverse sections of different duplex marginal teeth. Black horizontal line represents the subradular membrane; FIG. 23: *Comitas*-type; FIG. 24: Typical duplex tooth; FIG. 25: Semienrolled tooth of *Zonulispira*; FIG. 26: Semienrolled tooth of *Ptychobela*. Abbreviations: al – accessory limb; ct – central tooth; lt – lateral tooth; ml – major limb; vlt – vestigial lateral tooth.



FIGS. 27–32. Radulae of Clavatulidae. If not otherwise mentioned, data for the specimens are given in Table 2. FIGS. 27, 28: *Pusionella compacta* Strebel, 1914, MNHN IM200717830; FIG. 28: Enlarged central segment of the radula with vestigial lateral teeth; FIGS. 29, 30: *Clavatula xanteni* Nolf & Verstraeten, 2006, MNHN IM200717829; FIG. 30: Enlarged central segment of the radula. Arrow indicates the narrow central tooth; FIG. 31: *Toxiclionella tumida* (Sowerby, 1870), South Africa, after Kantor & Taylor (2000). Semi-enrolled marginal teeth. Left upper corner – diagrammatic section of the tooth. Subradular membrane was strongly damaged during the preparation of the radula; FIG. 32: *Gemmuloborsonia colorata* (Sysoev & Bouchet, 2001), MNHN IM200717849. Abbreviations: vlt – vestigial lateral teeth; ct – central tooth.



FIGS. 33, 34: Radulae of Turridae; FIGS. 35–38: Radulae of Pseudomelatomidae; FIGS. 39–41: Radulae of Cochlespiridae. All with different duplex marginal teeth. If not otherwise mentioned, data for the specimens are given in Table 2; FIG. 33: *Xenuroturrus legitima* Iredale, 1929, MNHN IM200717684; FIG. 34: *Turridrupa acutigemmata* (E. A. Smith, 1877), MNHN uncataloged, New Caledonia. Radula with narrow central and plate-like lateral teeth; FIG. 35: *Carinodrillia dichroa* Pilsbry & Lowe, 1932, NHMUK MOEA 20100530; FIG. 36: *Funa incerta* (Smith, 1877), NHMUK MOEA 20100554; FIG. 37: *Cheungbeia robusta* (Hinds, 1839), NHMUK MOEA 20100557; FIG. 38: *Inquisitor* sp., MNHN IM200717851; FIGS. 39, 40: *Cochlespira radiata* (Dall, 1889), MNHN, SE Brazil, after Kantor & Taylor, 2000; FIG. 41: *Sibogasyrinx* sp., MNHN IM200717701. Abbreviations: al – accessory limb of the marginal duplex tooth; ct – central tooth; lt – lateral tooth; ml – major limb of the marginal duplex tooth.

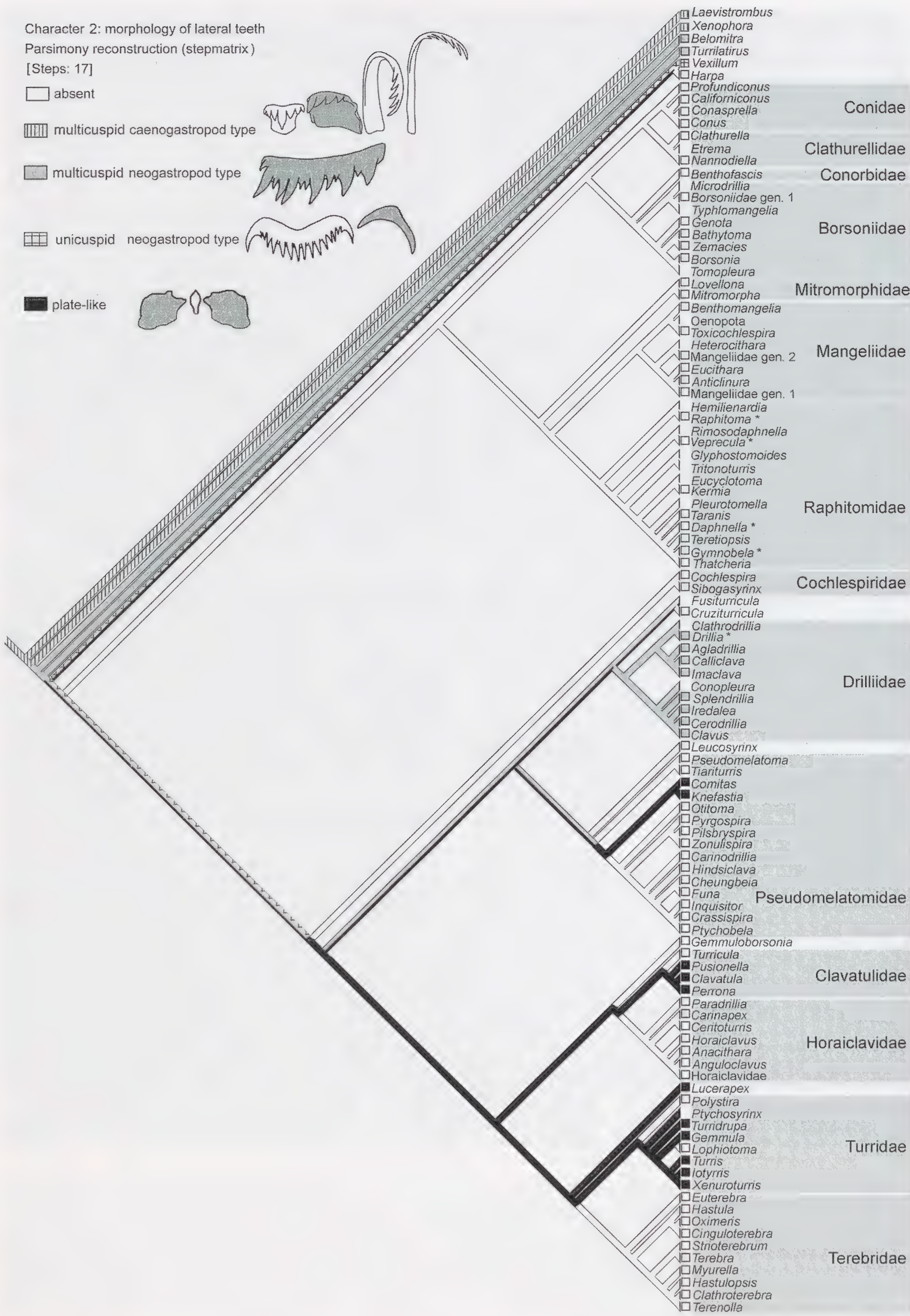


FIG. 42. Evolution of the lateral radular tooth morphology (character 2) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). A stepmatrix parsimony model was used, interdicting reversion of the character states.

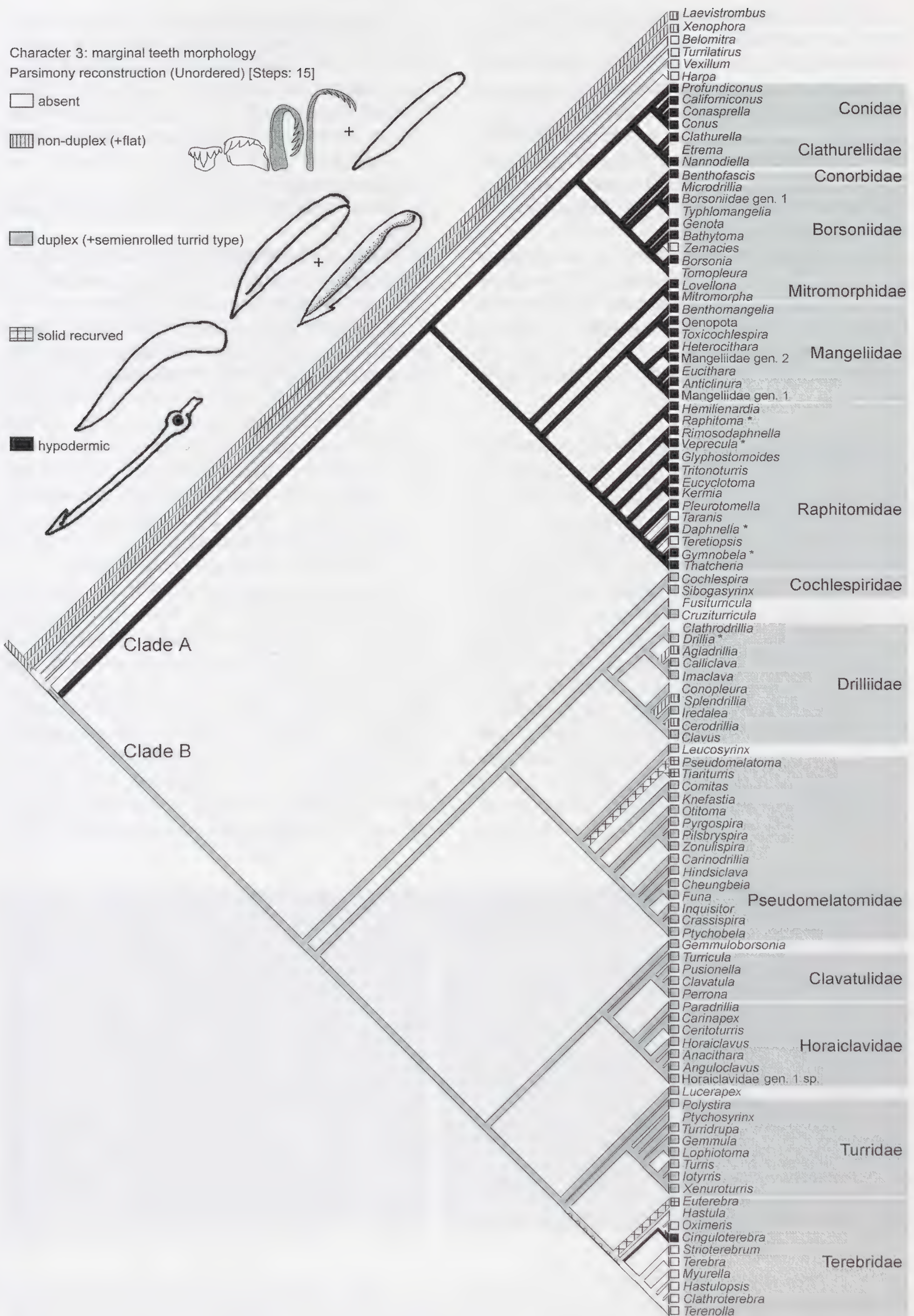


FIG. 43. Evolution of marginal tooth morphology (character 3) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered.

neogastropod type teeth and plate-like lateral teeth were equally parsimonious.

For most of clade B conoideans (except Cochlespiridae) the most parsimonious state was plate-like teeth, while multicuspid teeth seem to appear in the branch that combines Drilliidae and Pseudomelatomidae. Lateral teeth are independently lost in several lineages – in most species of Pseudomelatomidae, Horaiclavidae, Terebridae and others.

In all the species in our tree, the presence of the lateral teeth was combined with the presence of the central tooth, which is not the case for all conoideans (see discussion).

Character 3: Morphology of the Marginal Teeth (Fig. 43)

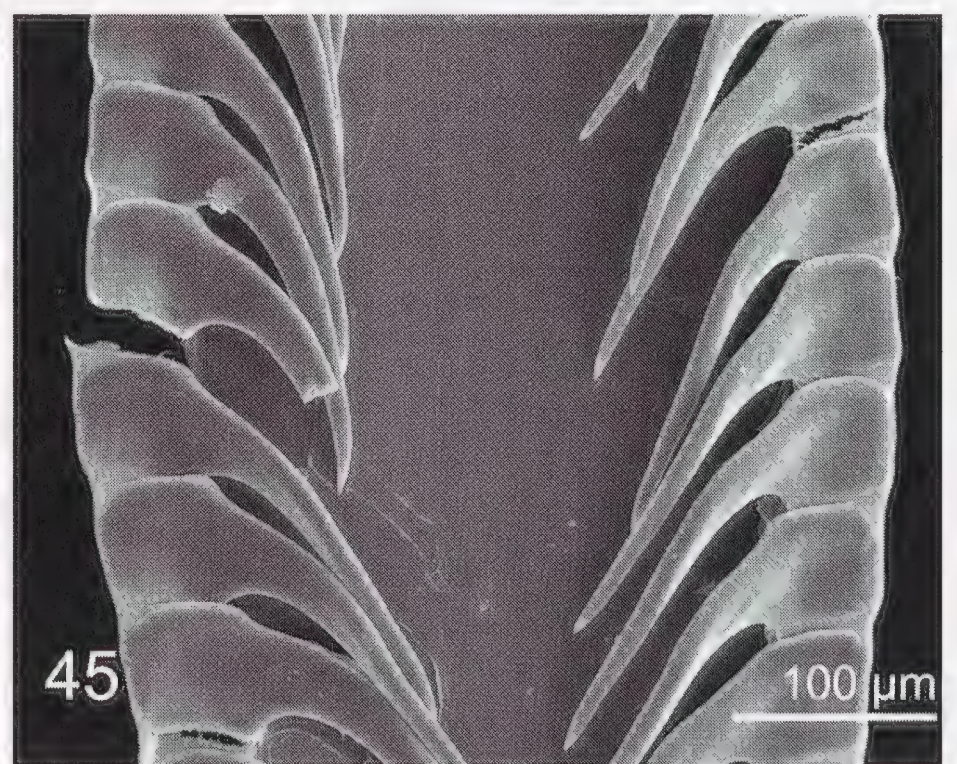
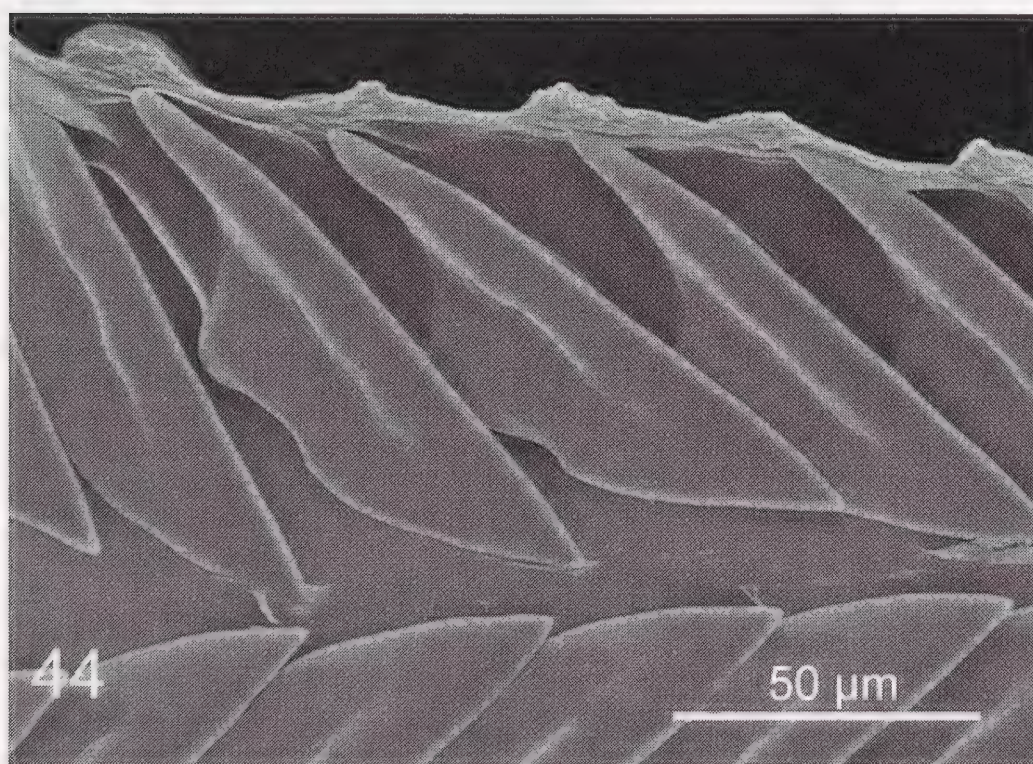
Despite great variability of the marginal teeth in Conoidea, four major types can be recognized:

- (a) duplex teeth, consisting of a major element (limb) attached to the subradular membrane along most of its length (Figs. 23, 24 – ml) and the accessory limb, that is the thickened edge of the major element, usually more or less elevated above the membrane (Figs. 23–25 – al). These teeth vary greatly in shape (Kantor & Taylor, 2000) (Figs. 27, 29, 32–41) and have often been referred to as “wishbone” (e.g., Powell, 1966). The term was coined based on the misconception that the limbs are separate and the tooth is actually bifurcating, as it appears under the light microscope (most of Clade B). In some cases, the limbs are

nearly equally developed and the teeth attain a trough-shape, becoming “semi-enrolled” (see below, Figs. 25, 26). In the analysis, this condition was also coded as “duplex teeth”. Very similar teeth were recorded in one species of Terebridae, *Pellifronia jungi* (Fig. 44), not present in our analysis (see below).

- (b) flat simple plate-like teeth (some Drilliidae) (Fig. 7).
- (c) solid, recurved teeth, attached to the membrane along part of the length, sometimes with a slightly broadened base that is actually attached to the membrane (some Pseudomelatomidae – *Pseudomelatoma*, *Hormospira* Berry, 1958, and *Tiariturris* Berry, 1958 – Fig. 14; some Terebridae – *Euterebra* and *Duplicaria* – Fig. 45, the latter not represented in our tree).
- (d) hypodermic teeth. These are hollow enrolled teeth attached to the subradular membrane only by a narrow base or through a flexible stalk, the ligament (some Borsoniidae, Conidae and others).

The analysis was not able to resolve the single most parsimonious state for the entire Conoidea, but suggested that a duplex tooth is the most parsimonious state for clade B. Flat teeth are characteristic only for some Drilliidae and according to the tree they are an autapomorphy of several species, thus suggesting their derivation from duplex teeth. Similarly, solid recurved teeth originated from duplex teeth twice independently in the evolutionary history of Conoidea – in some Pseudomelato-



FIGS. 44, 45. Radula of Terebridae with non-hypodermic teeth. If not otherwise mentioned, data for the specimens are given in Table 1. FIG. 44: *Pellifronia jungi* (Lai, 2001), MNHN 30591, Salomon 2, CP2195; FIG. 45: *Duplicaria bernardi* (Deshayes, 1857), Venus Bank, off NE end of Moreton Island, Moreton Bay, Queensland, Australia, 27°02'069"S, 153°19'00"E, 3.5–4.8 m, leg. Glover, Taylor, 2008.

midiae and Terebridae. Hypodermic teeth are a synapomorphy of clade A but also appeared independently in Terebridae. The marginal teeth have been also lost several times independently (at least three times in clade B and twice in Terebridae).

Character 4: Morphology of Duplex Marginal Teeth (Fig. 46)

Duplex teeth are very variable in morphology. The difference in appearance is mainly determined by relative size and shape of the accessory limb, as well as the degree of its elevation above the surface of the subradular membrane. The representation of the taxa in our tree does

not allow more detailed analysis, although the general patterns can be traced.

We recognize four subtypes of duplex teeth, although much more variation can be found in other Conoidea not included in our study.

The first subtype is characterized by equal or nearly subequal development of major and accessory limbs. This type of tooth is found in Cochlespiridae (Figs. 39, 40) (represented only by two genera in our tree) in which the teeth are characterized by relatively large size of the accessory limb (Fig. 40 – al) that is of nearly the same size as the major limb (Fig. 40 – ml). This produces the appearance of the tooth folded lengthwise. The analysis suggested that it is an apomorphy of the clade.

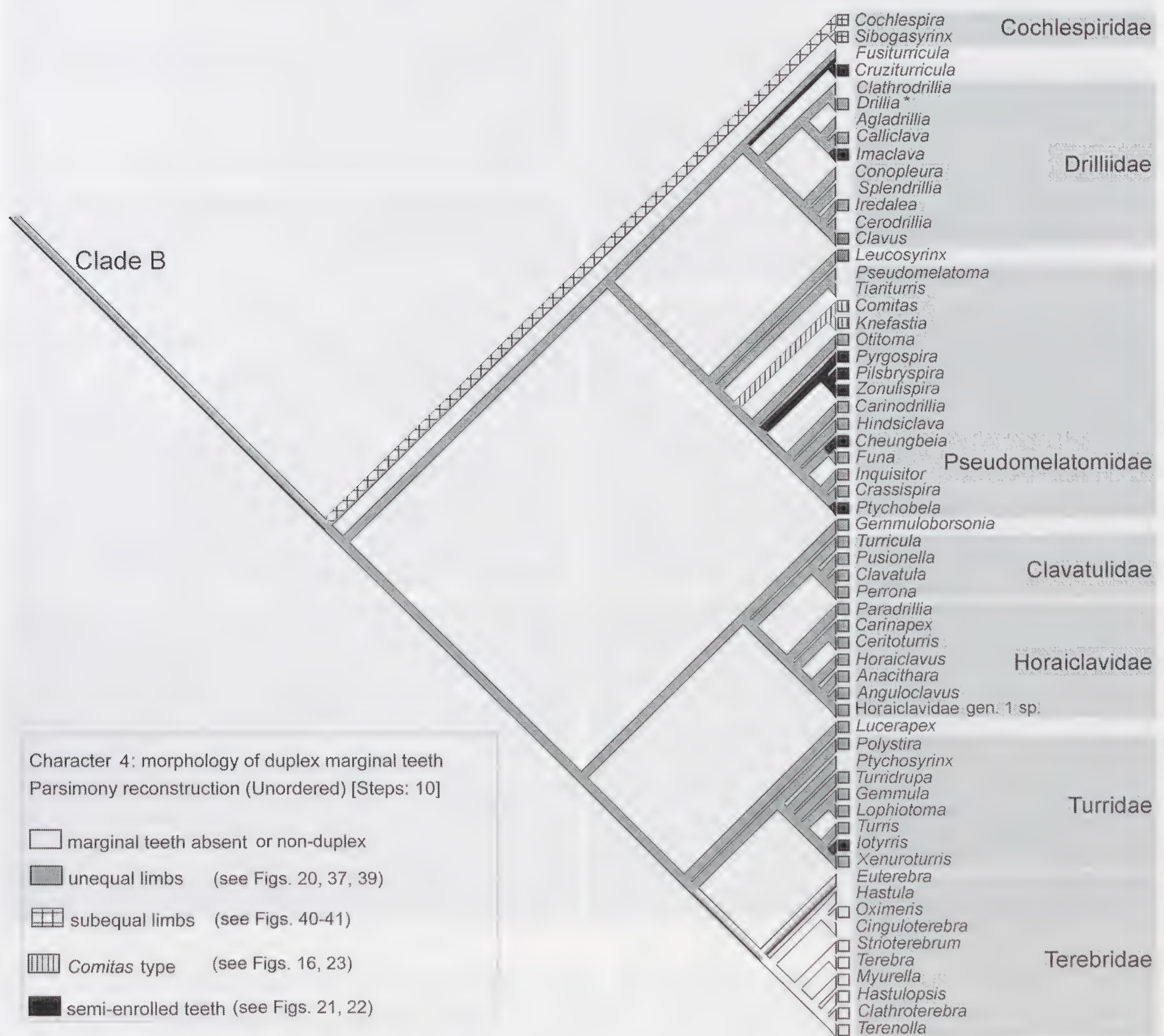
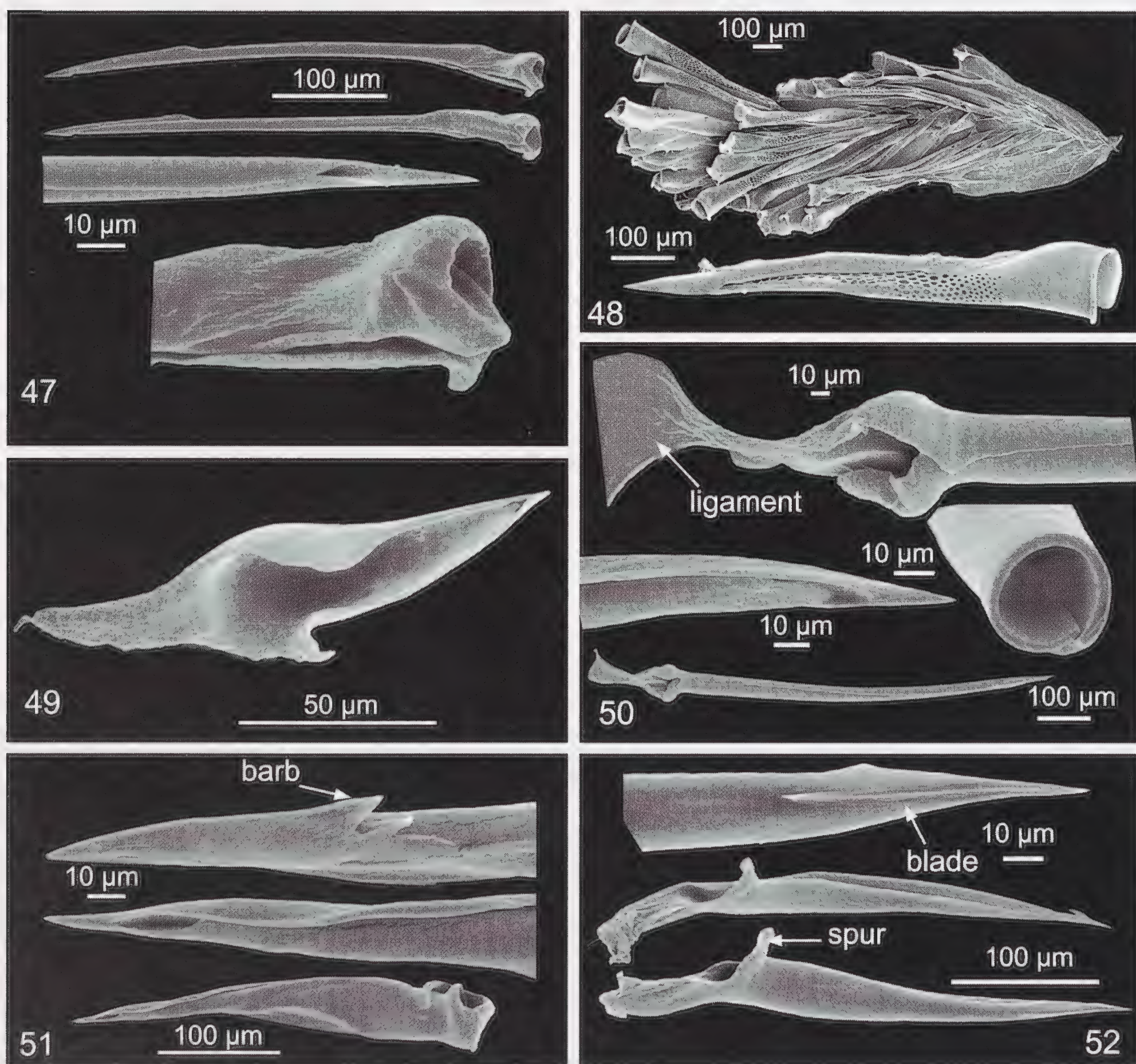


FIG. 46. Evolutionary transformations of duplex marginal tooth morphology (character 4) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. Since duplex marginal teeth are absent in the entire clade A, only clade B is illustrated.

A similar subtype, although having a different appearance, is the so-called semi-enrolled tooth (Taylor et al., 1993; Kantor & Taylor, 2000). In this type, the accessory limb is also subequal in size to the major limb (Figs. 25, 26), but the lengthwise folding is much less tight and the teeth attain a trough-like shape. According to the analysis, this type of tooth appeared several times independently in clade B – in some genera of Pseudomelatomidae (in the clade *Pilsbryspira* McLean, 1971, *Zonulispira*

Bartsch, 1950, and *Pyrgospira* McLean, 1971 – Fig. 21, and independently in *Ptychobela* Thiele, 1925 (Fig. 22), *Cruziturracula* Marks, 1951 (Fig. 11), *Imaclava* Bartsch, 1944, and *Iotyrris* (Turridae).

The most parsimonious plesiomorphic state for most of clade B (except Cochlespiridae) is the duplex marginal tooth with unequal sizes of the major, larger limb and smaller accessory limb (“unequal limbs” in Fig. 46). Depending on the degree of difference, the tooth may look



FIGS. 47, 48: Radula of Terebridae; FIGS. 49–52: Various hypodermic teeth from species of clade. If not otherwise mentioned, data for the specimens are given in Table 2; FIG. 47: *Cinguloterebra cingulifera* (Lamarck, 1822), MNHN 30565, Panglao 05, st. CP2340; FIG. 48: *Impages hectica* (Linnaeus, 1758), MNHN, uncataloged, Philippines, Panglao Island, Alona Beach, intertidal, 2004; FIG. 49: *Mangelia powisiana* (Dautzenberg, 1887). Plymouth, England, after Taylor et al. (1993); FIG. 50: *Bathytoma neocaledonica* Puillandre et al., 2010, MNHN IM200717857; FIG. 51: *Genota mitriformis* (Wood, 1828), MNHN IM200742293; FIG. 52: *Benthomangelia trophonoidea* (Schepman, 1913), MNHN IM200717835.

very different, in its most extreme state being nearly flat with a narrow and very slightly raised accessory limb (e.g., *Funa* Kilburn, 1988 – Fig. 36). In most groups, the accessory limb is comparatively large and the tooth edge adjoining the limb is significantly raised above the membrane, so that the accessory limb occupies the dorsal position on the major limb (e.g., 20, 38). Different teeth of this subtype have been thoroughly illustrated by Kantor et al. (1997) and Taylor et al. (1993).

A characteristic type of duplex tooth is found in the genera *Comitas* Finlay, 1926, and *Knefastia* Dall, 1919 (Figs. 16, 23). The teeth are nearly flat, broadly elongate, with the major

limb thickened at the tip and along one side, while the accessory limb is represented by the narrow thickened margin of the tooth that does not reach the tip of the tooth but is inserted in a shallow and narrow socket, slightly overlaying the thickened part of the major limb.

Several Characters (5–8) that Apply to Hypodermic Teeth Only (Figs. 47–52)

Hypodermic teeth are hollow enrolled marginals, usually with overlapping edges (exemptions are some representatives of Mangeliidae – Fig. 49, not present in our dataset) and open at both the tooth base and near the tip. Teeth

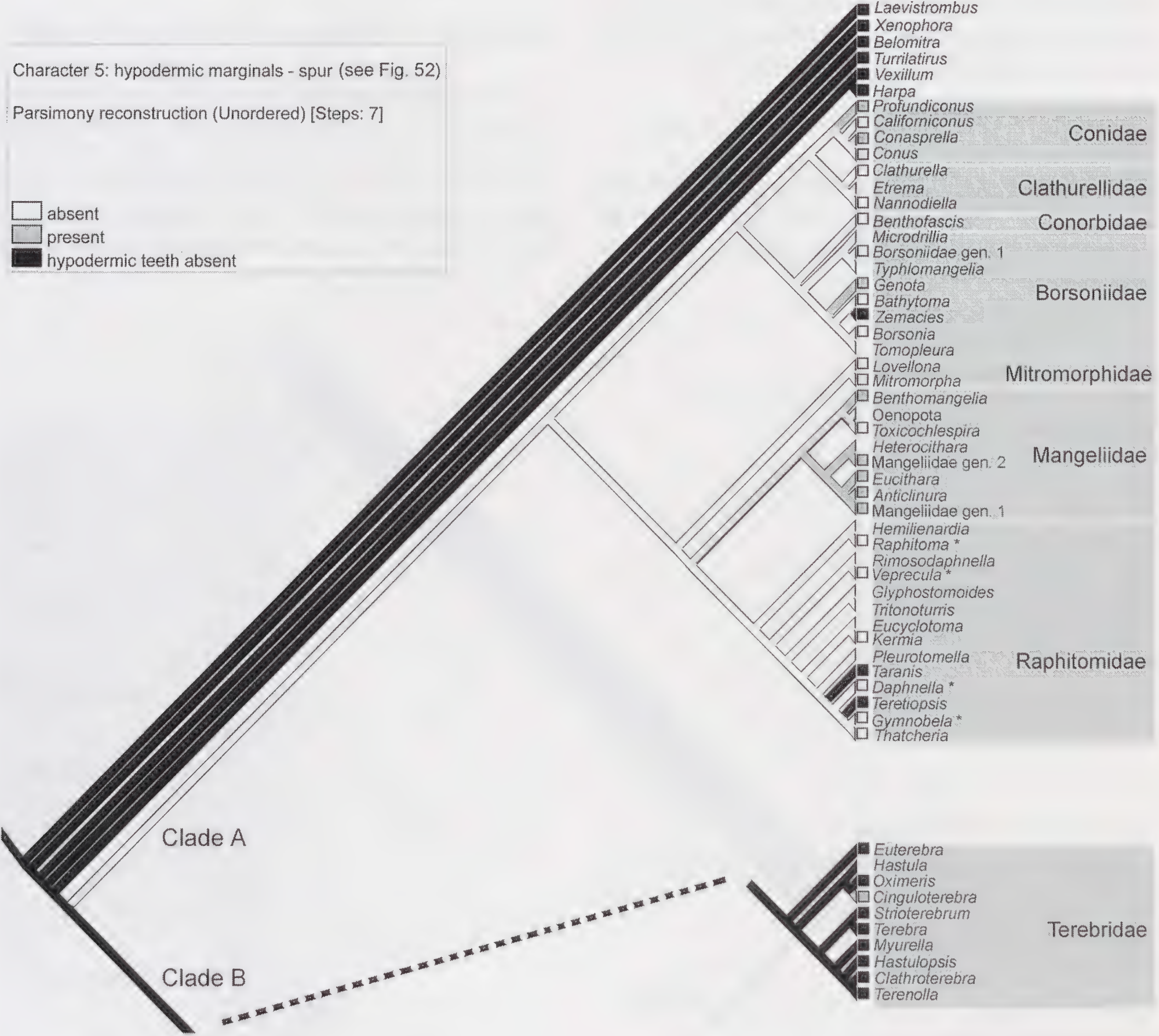


FIG. 53. Presence or absence of a spur on marginal teeth (character 5) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. The spur is absent in most of clade B, except some Terebridae. Therefore for clarity only Terebridae from clade B are shown.

of this morphology are found mostly among representatives of clade A and in most radulate Terebridae. Nevertheless, in at least one genus of Clavatulidae (*Toxiclionella* Powell, 1966) and in *Cruziturracula*, the marginal teeth are very similar in anatomy (Figs. 11, 31). The major difference between hypodermic teeth in clade A and Terebridae on one hand and in *Toxiclionella* and *Cruziturracula* on the other is the form of attachment to the radular membrane. In the former, the teeth are attached only by the base, while in the latter along most of their length. This suggests different evolutionary origins of such teeth (Kantor & Taylor, 2000).

High congruence was found between feeding type and hypodermic tooth anatomy in *Conus* (e.g., Duda et al., 2001; Espino et al., 2008). At the same time, there is a limited number of characters that are widespread across multiple families possessing hypodermic teeth.

Character 5: Presence of a Spur (Fig. 53)

The basal spur is an anterior projection on the base of the tooth (Fig. 52). Its function is probably to tighten the grasp of the proboscis

tip during feeding and thus to prevent premature loss of the tooth from the proboscis (Kohn et al., 1999). Our analysis suggested several independent origins of this character – in Conidae, Borsoniidae, Mangeliidae and Terebridae (Fig. 53).

Character 6: Presence of a Barb(s) (Fig. 54)

A barb is a projection from the shaft of the tooth that has a cutting edge and joins the shaft at an acute angle (Fig. 51). There can be from 0 to 5 barbs (*Conus californicus*) (Kohn et al., 1999). The analysis suggests that the barbs appeared independently in every family of clade A except Mitromorphidae, in which they are absent.

Character 7: Presence of a Blade (Fig. 55)

The blade is a projection from the shaft of the tooth that has a cutting edge and joins the shaft at an obtuse angle (Kohn et al., 1999) (Fig. 52). In some cases, the distinction between a barb and a blade is subtle. The analysis suggested that a blade originated independently twice – in

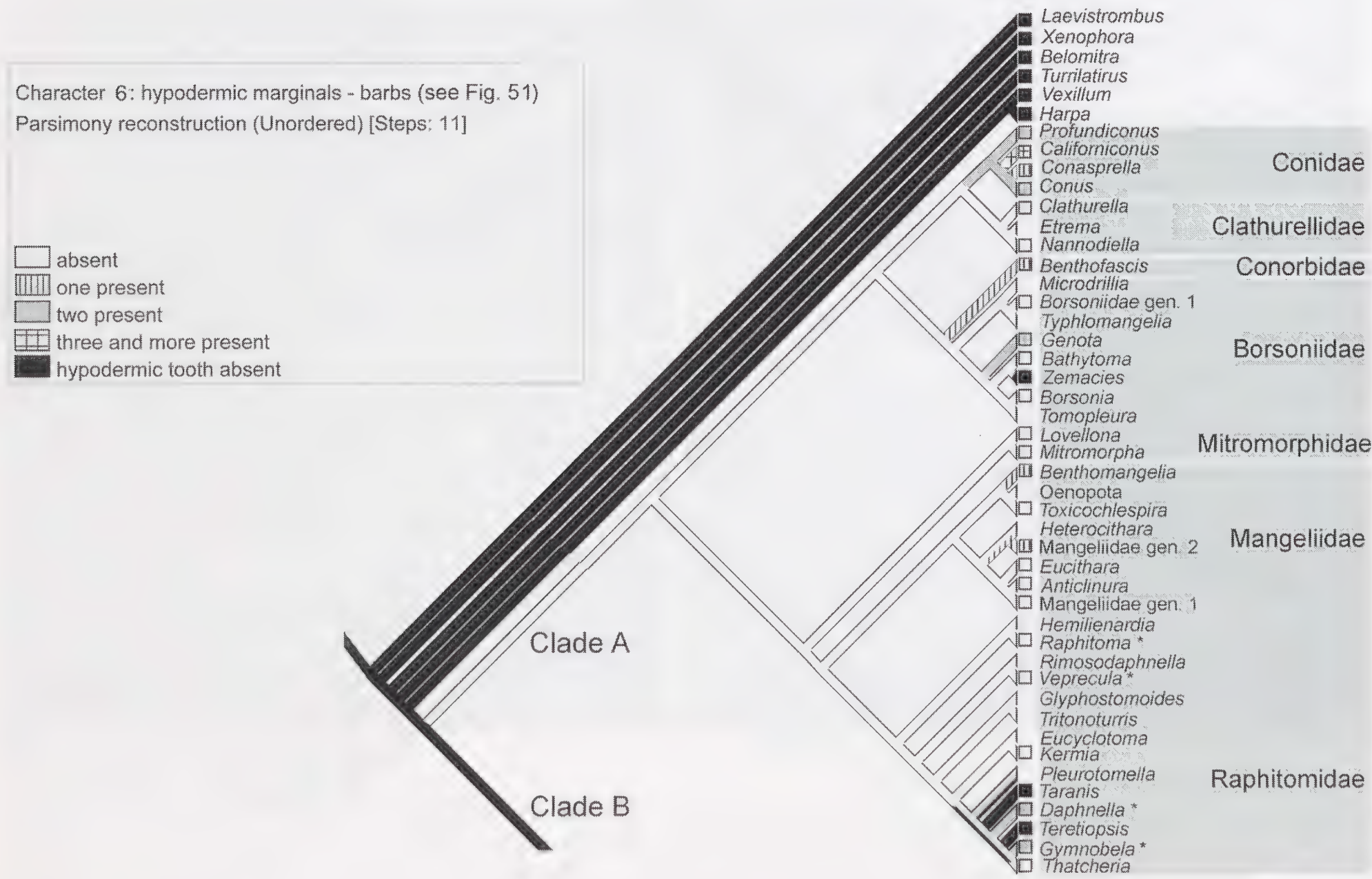


FIG. 54. Presence or absence of barbs on marginal teeth (character 6) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. The barb(s) are present only in clade A and therefore clade B is omitted.

clade A and in Terebridae. Presence of a blade was the most parsimonious ancestral state for clade A (Fig. 55).

Character 8: Presence of a Ligament (Fig. 56)

The ligament is an elongate, flexible stalk, attached to the base of the tooth and to the membrane, when the latter is present (Fig. 50). In fresh radulae, the ligament can be subcircular in cross-section, but when air-dried the ligament is usually flat and membrane-like. The presence of a ligament is often not recorded during radula description.

A ligament has so far only been recorded in clade A, and its presence is the most parsimonious ancestral state for the clade. It is present in Conidae, Borsoniidae, Mitromorphinae and at least in one species of Raphitomidae (*Thatcheria mirabilis* – see Taylor et al., 1993: fig. 23 c).

Character 9: Use of Marginal Teeth at the Proboscis Tip for Stabbing Prey (Fig. 57)

The analysis suggested that the use of marginal teeth at the proboscis tip is the most parsimonious plesiomorphic state for the entire Conoidea.

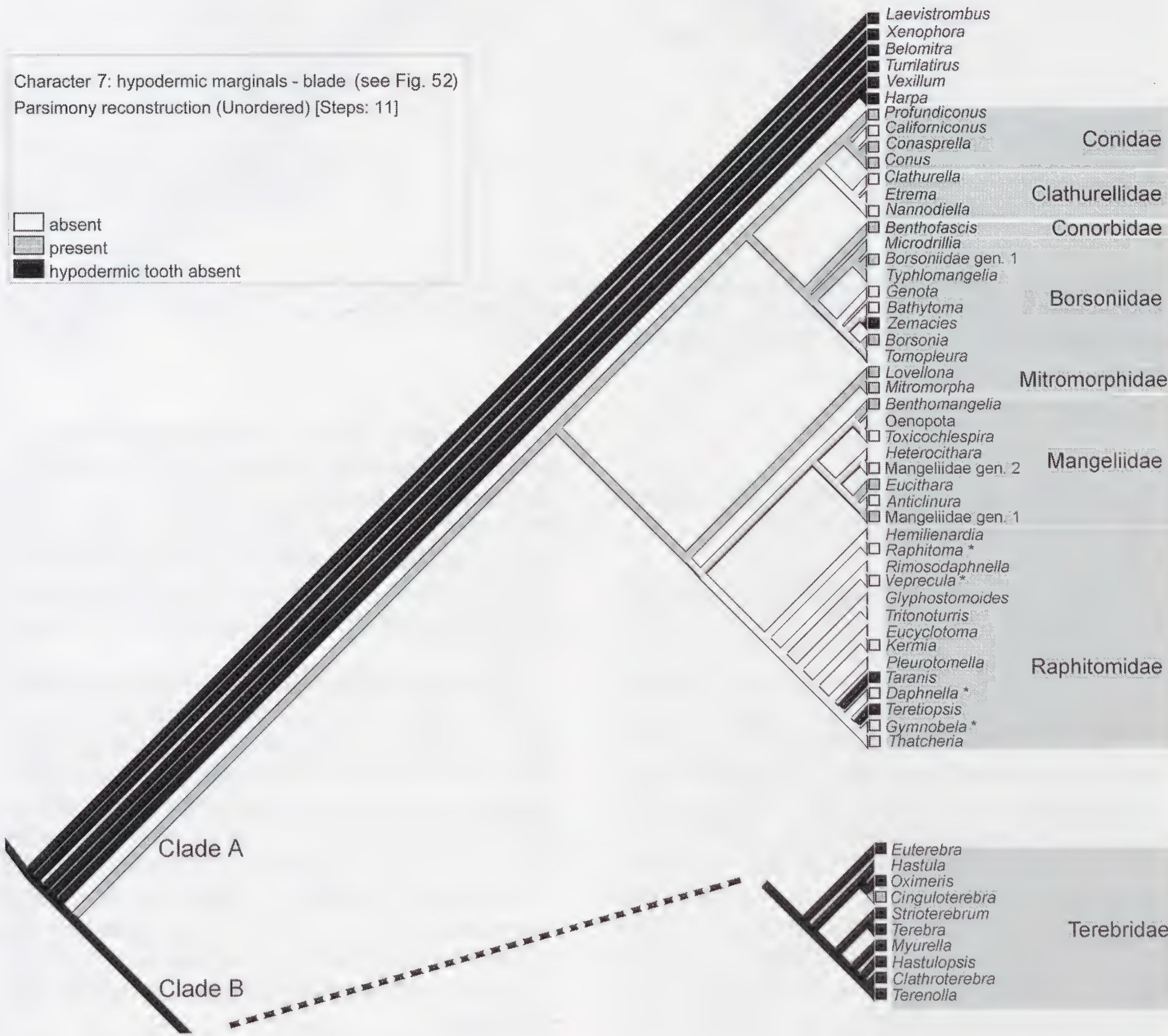


FIG. 55. Presence or absence of blade on marginal teeth (character 7) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. The blade is absent in most of clade B, except some Terebridae. Therefore for clarity only Terebridae from clade B are shown.

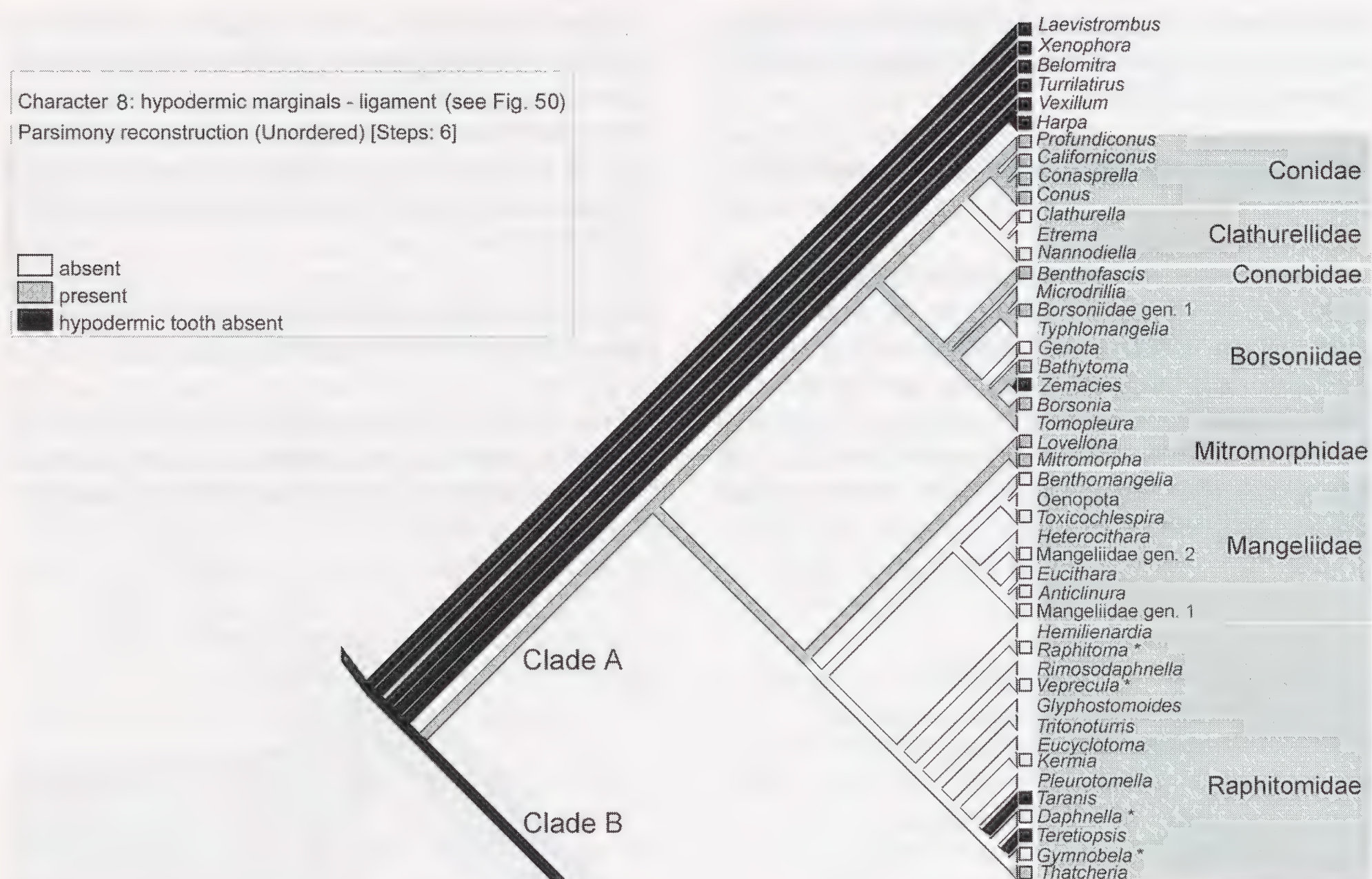


FIG. 56. Presence or absence of ligament of marginal teeth (character 8) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered. The ligament is present only in clade A and therefore clade B is omitted.

DISCUSSION

Origin of the Conoidean Feeding Mechanism and General Evolutionary Trends

Use of separate marginal teeth one at a time after their detachment from the rest of the radular apparatus at the proboscis tip is one of the most intriguing characters of conoidean evolution. Taylor et al. (1993) suggested that conoidean feeding mechanisms gradually evolved within the group, but appeared already at early stages of conoidean evolution. We traced on a molecular tree the possibility of use of individual marginal teeth at the proboscis tip using available published and unpublished anatomical data. As was mentioned in the introduction, the base of the tooth is held by special sphincter(s) and/or an epithelial pad of the buccal tube. Thus, use of teeth at the proboscis tip can be inferred from anatomical characters (presence of the sphincters of the buccal tube). Although we do not have anatomical data for every species included in our analysis, they are available for species of most of the genera and for every family, allowing us

to extrapolate to the remaining members of the clade. The analyses clearly suggested that contrary to the hypothesis of Taylor et al. (1993) the origin of the peculiar feeding mechanism was not a gradual process but is an apomorphy of Conoidea in general, and it appeared before the divergence of the two major clades (A and B) (Fig. 57).

The initial divergence of Conoidea into clades A with primarily hypodermic, and B, with primarily duplex marginal teeth is an unexpected inference from the conoidean molecular phylogeny. In previous cladistic analyses based on anatomical characters mainly of the anterior foregut, the representatives of clade A (referred to as family Conidae by Taylor et al., 1993) appeared as a terminal clade, suggesting the gradual transformation of radular morphology. The molecular-based results contradict this hypothesis.

The marginal teeth used one at a time after their detachment from the rest of the radular apparatus at the proboscis tip both in clades A and B and therefore there is no fundamental difference in feeding mechanism between these clades. Nevertheless, there are impor-

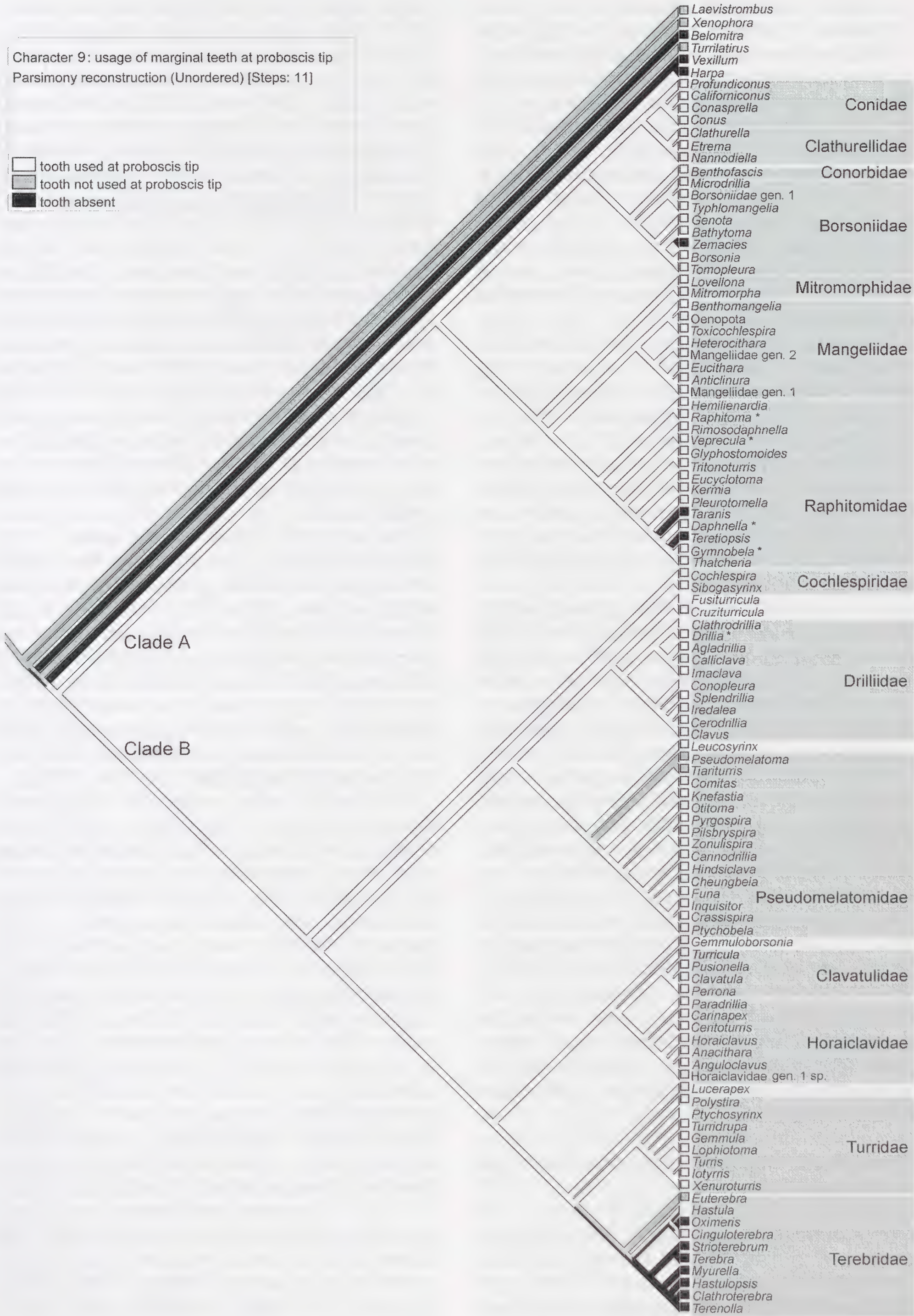


FIG. 57. Presence or absence of use of the separate marginal teeth on the proboscis tip (character 9) mapped on the conoidean molecular phylogeny (Puillandre et al., 2011). Character states are treated as unordered.

tant differences in the anatomy of the radular apparatus. In clade B, the radular apparatus consists of a more or less well-developed odontophore with supporting musculature (it is absent only in a few species that lack a radula, e.g., *Horaiclavus phaeocercus* Sysoev, 2008, Horaiclavidae – Fedosov & Kantor, 2008), moderately strong continuous radular membrane and (often) presence of central and/or lateral teeth. An important character of the radula is that the marginal teeth are attached to the membrane along a significant or even most of their length. Exceptions are some Terebridae (discussed below).

In clade A an odontophore with muscles is absent, and the subradular membrane is very thin to vestigial, although keeps the integrity of the radula in the radular diverticulum. The teeth are attached to the membrane only by the very narrow base of the tooth, sometimes through a flexible stalk – the ligament. The attachment of the marginal tooth (of hypodermic type) to the membrane only by the base facilitates rolling of the tooth, which may be formed by a few completely overlapping rolls in Conidae (for more details: Kantor & Taylor, 2000), although usually the edges of the tooth plate are only slightly overlapping (Fig. 50 – cross sections through the tooth). This attachment to the membrane only by the base of the tooth probably also facilitates the important process of complete separation of the tooth during feeding.

Thus, the molecular analysis suggests that appearance of the conoidean feeding mechanism was the key apomorphy of the superfamily. We suggest that it may have greatly improved prey capture and allowed rapid diversification and species radiation that resulted in the modern hyperdiverse group that includes about 4,600 Recent described species and a larger number of still unnamed taxa (Bouchet et al., 2009). The splitting of Conoidea into two groups with different radular types and foregut anatomies was the first major evolutionary event, taking place at the earliest stage of evolution of the group after the initial appearance of the unique feeding mechanism.

Transformation of the Non-Hypodermic Marginal Teeth (clade B)

Non-hypodermic (non-hollow) marginal teeth are found in clade B and are very variable, although they can be reduced to three major morphological types – duplex (including semi-enrolled), solid recurved and flat simple plate-like.

The solid recurved teeth were previously considered as the prototype marginal teeth in Conoidea (e.g., Kantor & Sysoev, 1990) and with the exception of three genera, which were united in the Pseudomelatomidae (*sensu* Taylor et al., 1993), were also found in some Terebridae (genera *Duplicaria* and *Euterebra*). Plotting the character on a molecular tree clearly indicated that this tooth type appeared independently twice in Conoidea. Analysis suggested that this type of marginal tooth developed secondarily from duplex teeth. Despite the general similarity of shape of the solid recurved teeth in Pseudomelatomidae and Terebridae, some important differences can also be mentioned. In Pseudomelatomidae *sensu* Taylor et al., 1993, the radula is long (about 100 rows of teeth in *Tiariturris*) and possesses large and broad, unicuspid central teeth; in Terebridae with these solid recurved teeth the radula is short (about 20 rows of teeth) and lacks the central teeth.

The functioning of this type of radula remains largely unknown. The shape of the rather strongly recurved teeth precludes their use one at a time at the proboscis tip. In *Duplicaria* and *Euterebra*, the venom gland as well as proboscis is absent, while the odontophore is present (Rudman, 1969; Taylor, 1990). In *Pseudomelatoma* and *Hormospira*, the venom gland is present and well developed, the proboscis is long, but the buccal tube lacks the sphincter that can hold a single tooth (Kantor, 1988).

The three genera comprising Pseudomelatomidae *sensu* Taylor et al., 1993 (and encompassing only six Recent species), have very limited distribution – all are found in the Panamic province. It is possible that they comprise a local radiation connected to a shift to some peculiar type of prey, although this needs further confirmation. The presence of a large odontophore suggests that radulae with solid recurved marginal teeth are primarily used as an entire organ (probably for tearing and rasping the prey). In contrast, Terebridae with this type of radula (Taylor, 1990) have a broad distribution in the Indo-Pacific – species are found from South Africa to Japan, including the Solomon Islands and Oman, and some species have a broad Indo-Pacific distribution. They lack a proboscis and venom gland. There are data for diet of only one species with this type of radula, *Terebra nassoides*, feeding on capitellid polychaetes (Taylor, 1990). Polychaetes of this family are widely distributed in the World Ocean.

Flat marginal teeth have also been considered as a prototype for the duplex teeth (Taylor et al., 1993) and were found in some Drilliidae (among the genera used in our analysis, in *Agladrillia*, *Splendrillia* and *Cerodrillia*). The analysis demonstrates that this condition is autapomorphic and this type of tooth originated by weakening and reduction of accessory limb of the duplex teeth, the presence of which is the plesiomorphic state for the entire clade B and Drilliidae in particular.

Within the most common duplex type of marginal teeth, many different morphologies can be recognized (Figs. 16–21, 33–41), although they are very similar in mode of formation. Kantor & Taylor (2000) studied maturing teeth along the radular membrane and showed that they develop from a flat plate by thickening of the tooth edges and elevation of the posterior edge (additional limb) from the membrane. This thickening of the margins of duplex teeth, folding along the length and partial enrolling (in the semi-enrolled teeth) can be explained as features that provide mechanical strength. In mechanical terms, a simple flat plate is less stiff and more likely to buckle when subject to a compressive force, than one with thickened edges or a hollow cylinder (Wainwright et al., 1976). Teeth used at the proboscis tip need to be rigid to pierce the prey's integument.

Although marginal tooth shape appeared to be rather homoplastic, some of the well-defined clades have characteristic tooth shapes. For example, in Cochlespiridae the accessory limb is large, nearly equal in size to the major limb. Therefore, the tooth appears to be folded lengthwise with a solid tip. Clavatulidae also possess rather distinctive duplex teeth with a sharp-edged major limb and a deep socket where an accessory limb is inserted, often with angulation distal to the socket. The well-supported clade including *Pilsbryspira*, *Zonulispira*, and *Pyrgospira* is characterized by semi-enrolled teeth with a similar shape and was previously considered a separate subfamily Zonulispirinae. In some duplex teeth (e.g., in *Funa* and *Cheungbeia*), the secondary limb is minute, nearly obsolete. Kantor & Taylor (2000) suggested, based on a morphological tree, that this is a derived state. The current analysis confirms this hypothesis.

In the single genus *Toxiclionella* Powell, 1966 (not present in our analysis), referred to Clavatulidae and that still possesses an odontophore, the teeth are hardly distinguishable from hypodermic teeth of the clade A (Fig. 31),

having two barbs at the tip and a subterminally opening tooth canal. Nevertheless, contrary to hypodermic teeth of the species of the clade A, the marginal teeth in *Toxiclionella* are attached to the membrane along most of their length, similarly to other species of clade B.

Transformations of the Central Radular Segment (Clade B)

In all Conoidea, the buccal mass with the radular diverticulum (and odontophore with muscles in clade B) is situated at the proboscis base and often behind proboscis in its contracted state. The odontophore cannot be protruded through the mouth and therefore the radula can function in most conoideans as an integrated whole organ only in the buccal cavity, but not for grasping and tearing the prey. A few exceptions occur sporadically in different clades. In these groups, the buccal mass is secondarily shifted anteriorly or is able to evert through the mouth along with the walls of the buccal tube (e.g., in *Funa latisinuata* – Taylor et al., 1993: fig. 14).

The limited functioning of the radula as an integrated organ in adults is indirectly confirmed by absence of worn teeth (marginal, and central or laterals when present) in radulae examined by us (except Drilliidae, see below). Odontophore in general is rather small (sometimes its presence can be confirmed only on serial sections), with poorly developed muscles. Thus, the central and lateral teeth are hardly functional in Conoidea (while the marginals detached from the membrane are used individually at the proboscis tip) and therefore the adaptive value of morphological transformations of central and lateral teeth may be reduced. This explains the high variability of the morphology of the central segment of the radula that includes the central and lateral teeth.

The evolutionary transformation of the central (and lateral teeth) in clade B is complicated. Analysis of the morphology of the central tooth with unordered character states produced a shorter tree, but suggested initial reduction of the tooth in the entire Conoidea and subsequent multiple (eight in our dataset) independent reappearances in different clades, that is multiple reversions (Fig. 5). It is especially difficult to explain these reversions from a functional point of view due to the limited functions of the central and lateral teeth. On the contrary, reduced functionality of the central segment seems to be congruent with reduction

and complete loss of the central segment in different lineages, as was suggested by the stepmatrix analysis (Fig. 6).

Similarly, we found the numerous losses of the lateral teeth (stepmatrix analysis – Fig. 42) more probable (although less parsimonious) than initial reduction of the teeth in the entire Conoidea and independent reappearance in five clades (analysis with unordered character states). Only in Drilliidae do the lateral teeth seem to be functional (see below), while in other clades they are very weak plate-like structures, sometimes appearing only as inconspicuous thickenings of the subradular membrane (e.g., in *Pusionella compacta* – Fig. 28, vlt).

The central tooth is thought to be highly variable in Conoidea, ranging from very narrow unicuspid to broad with a large cusp and well-developed lateral flaps. Kantor & Sysoev (1991) and Taylor et al. (1993) suggested that the broad central tooth in some groups may be the result of fusion of paired lateral teeth with the narrow unicuspid central tooth. At that time, conclusion was rather hypothetical and besides general shape of the tooth was based on the pattern of radular membrane folding. In some species, attributed here to Pseudomelatomidae (*Crassiclava turricula* – Figs. 19, 20, *Antiplanes sanctioannis* (Smith, 1875) – Kantor & Sysoev, 1991: figs. 27, 28; *Comitas onokeana vivens* Dell, 1956 – Fig. 15), clearly separate, plate-shaped lateral teeth without cusps were found. In these species, the central tooth (cusp) is absent. Later, in *Comitas* Finlay, 1926, and the related genus *Knefastia* Dall, 1919, intermediate stages were found. In *K. tuberculifera*, in addition to a very weak and reduced central tooth (Fig. 18, ct) vestigial lateral plates (teeth) can be observed (vlt); in *Comitas pachycercus* Sysoev & Bouchet, 2001, *Comitas murrayi* (Garrard, 1961), and *Comitas* sp. (Figs. 16, 17), the central structure looks like a well-defined central tooth with a narrow cusp and broad lateral flaps (no lateral teeth present). In our opinion, this transitional row suggests that instead of a morphologically extremely variable central and lateral teeth in closely related groups, we are observing the different stages of fusion of lateral cusplike plates with narrow central tooth. Similar gradual stages of fusion of lateral and central narrow tooth was demonstrated in Turridae, in which transitional conditions ranging from a clearly tripartite structure with a gap between the cusp (= central tooth) and the lateral flaps (= lateral

teeth) to a seemingly solid central tooth occur, sometimes within a single genus, for example, *Gemmula* (Kantor, 2006).

In three clades – in Cochlespiridae, in clade combining *Pseudomelatoma* and *Tiariturris* (Pseudomelatomidae *sensu* Taylor et al., 1993), and in *Gemmuloborsonia*, there is no indication that the central tooth has a composite origin. The posterior margin bearing the cusp is equally developed along its width and elevated over the radular membrane. Without further information, we conclude that these groups possess the broad unicuspid central tooth, while the lateral teeth are absent.

Thus, the great morphological variability of the central segment of the radula in all clades (except the three mentioned above) is likely to correspond to the different stages of the process of fusion of lateral and central teeth with simultaneous reduction of either central, or lateral or both teeth.

One of the unexpected results of our analysis is the possible secondary origin of the multicuspitate separate and well-formed lateral teeth in Drilliidae. Previously, this type of the tooth was considered prototypic for turrids (Powell, 1966; Kantor & Sysoev, 1991), and this hypothesis was the rationale for placing the Drilliidae as a separate family from the other Turridae *sensu* Taylor et al. 1993. According to the analysis, the multicuspitate lateral teeth may be the ancestral state for clade B; they then disappeared and reappeared again in the Drilliidae plus Pseudomelatomidae clade, though multicuspitate teeth are present only in the former family. However, different runs of the analysis with different coding of radular character states in the outgroups suggested different most parsimonious plesiomorphic conditions in clade B, but in none of the runs were multicuspitate teeth the single plesiomorphic state. One of the reasons for these inconclusive results may be the fact that the homology of different teeth in Neogastropoda (including outgroups) is not yet finally established and the relationships within the entire Neogastropoda are far from resolved. Therefore, the sister group of Conoidea is not yet identified, and we do not know what is the plesiomorphic morphology of the conoidean forerunner.

From a general point of view, it seems more probable that well-pronounced multicuspitate lateral teeth in Drilliidae is the plesiomorphic condition in Conoidea, retained due to peculiarities of their feeding mechanism. Unfortunately, there is practically no information on the diet

and radula functioning of drilliids. The only published record is that of Maes (1983) of the gut content of *Drillia cydia* (Bartsch, 1943). Finding intact prey (sipunculid) in the posterior oesophagus suggested that the radula is used for gripping and/or piercing, not rasping or tearing. Drilliidae studied by us possess large and powerful odontophores, which may suggest active use of the radula as an integrated whole organ. To confirm this, we carefully examined the bending plane of the radulae. In many species the laterals and even the central teeth were badly damaged (Fig. 12 – broken parts of the teeth are marked by small arrows); damage on the marginal teeth in the same specimens was not observed. These observations suggest that lateral (and even small central) teeth are functional, but without additional data on feeding in this group, it is impossible to draw any final conclusions about the mode of functioning.

One important question remains: why in clade B conoideans, which possess in general the same feeding mechanism as clade A species, are the odontophore and its musculature retained? The odontophore varies in size from large in Drilliidae and some Pseudomelatomidae (*Pseudomelatomia*) (Taylor et al., 1993) to very small or nearly obsolete (e.g., in some *Hindsiclava*, Pseudomelatomidae – Kantor et al., 1997). There are no data that explain this phenomenon. From what has been said above, it is clear that the functioning of the radula as an integrated organ may be limited, possibly to transferring the swallowed prey from the buccal cavity, situated at the proboscis base, further to oesophagus. Only in very few conoideans is the buccal cavity plus radula either shifted to the proboscis tip or able to protrude through the mouth due to the eversion of the buccal tube (for details: Taylor et al., 1993). From the point of view of speciation, clade A conoideans are more diverse, including 202 genera (not counting 82 genera recognized in the Conidae, the phylogeny and taxonomy of which is not yet finally revised) versus 180 genera in clade B. Thus, absence of the odontophore does not seem to limit prey capture and feeding. Moreover, clade A conoideans seem to have a broader prey range, including other gastropods (numerous *Conus* species) and bivalves (*Phymorhynchus*, Raphitomidae – Fujikura et al., 2009) and even fish (several species of *Conus*).

In some species of *Conus*, ontogenetic changes of radular teeth are probably related to changes in diet (summarized by Nybakken,

1990) and therefore in prey capture mechanism. We may imagine that similar changes can occur in clade B and that the prey capture and feeding mechanisms may differ between young individuals and adults and that at some ontogenetic stage the odontophore may be fully operational. This supposition needs careful research to detect any ontogenetic radular and foregut anatomy changes.

Transformation of the Hypodermic Marginal Teeth

Hypodermic marginal teeth are found in clade A and some Terebridae. The morphology of the hypodermic teeth is extremely variable and was traditionally used for taxonomy. Some hypodermic teeth are very simple, semi-enrolled. Such teeth are found among Mangeliidae (e.g., *Mangelia* – Fig. 49). Unfortunately, none of these species is present in our molecular tree.

The hypodermic teeth of Conidae s.s. have been described in great detail and correlation between tooth morphology and diet has been demonstrated (e.g., Nybbaken, 1990). The representation of genera in our data matrix is relatively sparse and therefore we could only trace a limited number of characters of the hypodermic teeth. It appeared that the spurs and barbs of the teeth are homoplastic and evolved independently several times. No clear trends were obvious, possibly due to the incompleteness of our dataset.

Terebridae Radiation

One of the most remarkable findings of the molecular analysis (Puillandre et al., 2008, 2011) is that the Terebridae do not represent a totally separate lineage, but are included in clade B and are sister to the Turridae.

Common among all terebrids is complete loss of the central and lateral teeth. In our analysis, the first taxon to diverge among the Terebridae is *Euterebra tristis* (Deshayes, 1859), characterized by the solid recurved teeth, superficially similar to that in *Pseudomelatomia* and related genera (Pseudomelatomidae). Our analysis was not able to resolve the most parsimonious ancestral state for Terebridae. Before the molecular analysis of all Conoidea was performed (Puillandre et al., 2011), more detailed analysis of Terebridae has been conducted (Holford et al., 2009). Among other things, it revealed that *Pellifronia jungi* (Lai, 2001) might constitute sister lineage to all the other Terebridae. The radula of *P. jungi* appeared to have a new

type of marginal teeth for the family, similar in general arrangement to the duplex teeth of other families in clade B (Fig. 44). The species possess a venom gland with a muscular bulb, proboscis, and small odontophore.

Thus, within the Terebridae, the entire transition can be found from species with duplex teeth with a strong subradular membrane (and an odontophore) through solid recurved teeth to species with typical hypodermic teeth, attached only by their bases to a vestigial membrane (similar to the arrangement of the marginal teeth in clade A and similarly lacking an odontophore). There are some species (not included in our analysis, but the position of which was inferred in the molecular phylogeny of Holford et al. 2009), for example, *Impages hectica* (Linnaeus, 1758), that possess hypodermic teeth (penetrated by numerous holes – Fig. 48) that are attached to a rather strong membrane along their length, a condition similar to that in *Toxiclionella* (Clavatulidae).

Within this single clade the radula transformation independently parallels the evolution of the radular apparatus within the entire Conoidea. This is a remarkable example of the radular evolvability in Conoidea.

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THE PHYLOGENY AND MORPHOLOGICAL ADAPTATIONS OF *CYCLOTUS TAIVANUS* SSP. (GASTROPODA: CYCLOPHORIDAE)

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ABSTRACT

By traditional classification, there are five *Cyclotus taivanus* subspecies in the low mountainous area of Taiwan and Okinawa: *C. taivanus adamsi*, *C. t. dilatus*, *C. t. diminutus*, *C. t. peraffinis*, and *C. t. taivanus*. The molecular phylogenetic relationships of this group have never been discussed. In order to investigate the relationships between *C. taivanus* ssp., we sequenced part of the mitochondrial COI and the 16S rRNA gene from 26 sampling sites. We also measured 9 shell traits for morphological analysis. Even though morphological PCA analysis revealed a more or less continuous distribution of individuals in morph-space, the two highly divergent haplotype clades in molecular analysis indicated the presence of two independently evolving lineages. Our results indicated that the sequence divergence between the two independent clades was almost as high as that among other Cyclophoridae species found previously. Therefore, from the viewpoint of taxonomy, *C. t. adamsi* should be considered a valid species, and we here raise the current taxon to a full species: *C. adamsi*. By environmental analysis, temperature was found to be a limiting factor in the distribution of *C. adamsi* and the *C. taivanus* group (*C. t. dilatus*, *C. t. diminutus*, *C. t. peraffinis*, and *C. t. taivanus*). The ecological divergence is probably a rule of speciation in our case. The PLS (Partial least square) analysis results indicate that phenotypic plasticity may be a key element of the variable shell in the *C. taivanus* group. The speciation process is not complete among the *C. taivanus* group, and the adaptation to climatic pressure continues to be a rule of the speciation process.

Key words: phylogeny, morphological adaptations, *Cyclotus taivanus*, biogeography, Taiwan.

INTRODUCTION

Cyclotus taivanus ssp. is widely distributed in Taiwan and the Japanese island of Okinawa. Traditionally, five subspecies have been described. The north Taiwan subspecies *C. t. adamsi* (Pilsbry & Hirase, 1905) has a tall spire with a uniform yellow or tawny yellow color, although some individuals show a reddish-brown zigzag pattern. The other four subspecies have flat spires, but are characterized by a wide extended lip in the east Taiwanese subspecies *C. t. dilatus* Lee & Wu, 2001, a very narrow lip in the Lanyu Island subspecies *C. t. diminutus* Lee & Wu, 2001, an intermediate lip in the south Taiwanese subspecies *C. t. taivanus* H. Adams, 1870, and a very polished periostracum in the Okinawan subspecies *C. t. peraffinis* (Pilsbry & Hirase, 1905) (which is otherwise similar to *C. t. diminutus*) (Lee & Wu, 2001).

Although, some microsnails can potentially achieve long-distance colonization through

passive dispersal (Kirchner et al., 1997; Wada et al., 2011), most land snails have a low vagility (Anderson, 2007; Murray & Clarke, 1984; Thomaz et al., 1996), and therefore cannot avoid unfavorable conditions by migration but are doomed to extinction (Akçakaya & Baur, 1996). Landslides and debris flows often occur in Taiwan due to heavy orogenic movements, the fragile geological environment, earthquakes, typhoons and heavy rainfall (Lin et al., 2004; Yeh, 2006). The snails may isolate in relative stable microhabitat and raise their genetic variance. These render land snails an ideal model for examining the role of speciation history in our place.

The evolutionary processes shaping the phenotypic variation among and within related landsnails have recently become a focus of research interest (Booth et al., 1990; Gould et al., 1974; Gould & Woodruff, 1978; Pfenninger et al., 2003; Pfenninger & Magnin, 2001; Teshima et al., 2003; Woodruff & Gould, 1987). Two ba-

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sis approaches have been taken to the study of variation in shell shape. One approach involves analysis of the distribution of shapes among taxa and hypothesizing about the causes affecting the frequencies of certain shapes (Raup, 1966), while the second approach involves study of the correlations between variation in shape and environmental variation (Goodfriend, 1986). In earlier times, biologists and taxonomists discriminated species based on anatomy and morphology. On the beginning of the 1970s, molecular techniques have been used as an aid for delimiting species (Michael, 1996). Mitochondrial DNA has an important advantage in phylogeny reconstruction and species delimitation (Wiens & Penkrot, 2002). However, malacologists have lagged behind other systematists in applying molecular techniques for delimiting species (Davis, 1994). The last decade, mtDNA data sets have been widely used as markers for delimiting mollusc species (Collin, 2005; Lapègue et al., 2002; Malaquias & Reid, 2008; Meyer, 2003). Further, the phylogeny and evolutionary processes of *C. taivanus* ssp. have not been investigated previously.

The objectives of this study were to use nucleotide sequence information from COI and 16S rRNA gene fragments to investigate the quantitative morphological differences between the subspecies of *C. taivanus*. In particular, we focus on four questions: (1) Does the observed phenotypic variation correspond to different evolutionary lineages? (2) How is the phenotypic variation distributed within and among populations in relation to the variations in COI and 16S? (3) Can we identify environmental variables that co-vary with population differences in morphology? (4) What is the cause of the speciation process?

MATERIALS AND METHODS

Populations Sampled

In total, 26 populations were examined in Taiwan (Fig. 1; Table 1). We did not obtain any living specimens (sampling site 2) or adult individuals (sampling site 5, 18) at some sampling sites. Morphological analysis was performed on 105 adult individuals from 24 locations and DNA analysis on 87 individuals from 25 locations. Both data sets overlapped for 70 individuals.

Morphological Analysis

Individuals were photographed using a Canon D1 digital camera from the aperture view, and eight shell characters were measured using ScopePhoto software (ScopeTek, 2007): H (height), W (width), AW (aperture width), AH (aperture height), BW (body whorl width), BH (body whorl height), LW (outer lip width), UW (umbilicus width) and HN (whorl number) (Fig. 2). $H \times W$, H/W , AW/W , AW/AH , UW/W , LW/AW , BH/BW and $(W-UW-LW)/HN$ were calculated as indices of size, tallness/flatness, aperture proportion, aperture shape, umbilicus proportion, outer lip proportion, flatness degree of body whorl and degree of whorl increase, respectively. These shell morphological variables and sampling site environmental characterization (warm season mean temperature, cold season mean temperature, annual mean temperature, annual range of monthly normal temperature, warm season amount of precipitation, cold season amount of precipitation, annual amount of precipitation, altitude, obtained from Taiwan Central Weather Bureau) (Lin, 1990) were used to perform principal component analysis (PCA) using the PCA option of the XLSTAT package (2010).

PLS analysis was performed using the PLS option of the XLSTAT package (2010) to assess the correlations between quantitative shell traits and environmental variables for each population.

DNA Preparation and Sequencing

The shells and soft parts were separated at the laboratory. Shells were cleaned thoroughly for identification and measurement of shell characters, and soft parts were stored at -80° until DNA extraction. DNA was extracted from the columellar muscle of separate individuals using a slightly modified TEK-based protocol (Jiang et al., 1997). Tissue was placed in TEK buffer (12.5 mM Tris-HCl pH 7.3, 2.5 mM EDTA, 0.4% KCl), then ground with a glass pestle and incubated at 57°C with 20 μl of proteinase K (20 mg/ml) for more than 2 hrs. Tissue was extracted at least twice with phenol and chloroform. 400 μl DNA extract was precipitated by adding 1,000 μl pure ice-cold ethanol and was stored at -20°C for 20 min. DNA was then pelleted by centrifugation for 30 min. After rinsing with 70% ethanol, DNA was resuspended in distilled water and stored at -80°C for DNA amplification.

TABLE 1. List of *Cyclotus* sampling sites used in this study.

	Location	Position	Morphological analysis	DNA ananlysis
1	Guan-in Mountain, Taipei County	25.135861N, 121.428861E	1	1
2	Chih-shan-yin, Taipei City	25.104833N, 121.529889E	3	—
3	The Cao-ling Historic Trail, Iran County	24.993194N, 121.925639E	1	6
4	Kai-cheng Temple, Iran County	24.813139N, 121.710583E	5	7
5	Sheau-jiau-shi, Iran County	24.805833N, 121.704417E	—	2
6	Fwu-shan, Taipei County	24.795750N, 121.492944E	3	1
7	Ren-tzer, Iran County	24.545611N, 121.507278E	1	1
8	Dung-aw-li, Su-aw, Iran County	24.521194N, 121.824889E	5	4
9	Iriomote Island	24.349000N, 123.805000E	5	3
10	Chorng-der, Hualian County	24.189278N, 121.660889E	10	9
11	Chin-heng Bridge, Taroko valley, Hualian County	24.171167N, 121.560417E	2	1
12	Charn-guang Temple, Taroko valley, Hualian County	24.159250N, 121.606111E	2	1
13	County Highway No. 136, 39.5K, Taichung County	24.099361N, 120.790389E	1	1
14	County Highway No.129, 20.5k, Jia-yi County	23.342500N, 120.691861E	7	8
15	Tu-di-gong Temple, Guan-tzy-liing, Tainan County	23.339306N, 120.505556E	5	7
16	Provincial Highway No.20, 49k, Nan-huah, Tainan City	23.100389N, 120.481556E	1	1
17	Shuang-liou Wood Park, Ping-dung County	22.217889N, 120.803000E	1	2
18	Jang-hu, Jia-yi County	23.599333N, 120.656472E	—	1
19	Provincial Highway No.149, Yun-lin County	23.589639N, 120.569306E	2	1
20	Bau-Lai, Kaohsiung County	23.108639N, 120.699722E	21	3
21	Shan-ping, Kaohsiung County	22.965917N, 120.683944E	7	9
22	Liou-guei, Kaohsiung County	22.925222N, 120.652833E	6	1
23	Hae-shern Temple, San-dih-men, Ping-dung County	22.819972N, 120.640444E	2	3
24	Wu-tai, Ping-dung County	22.751528N, 120.727167E	1	1
25	Wu-tai, Ping-dung County	22.743167N, 120.706944E	3	3
26	Yongsing, Lanyu Island, Taidung County	22.028222N, 121.579667E	10	10

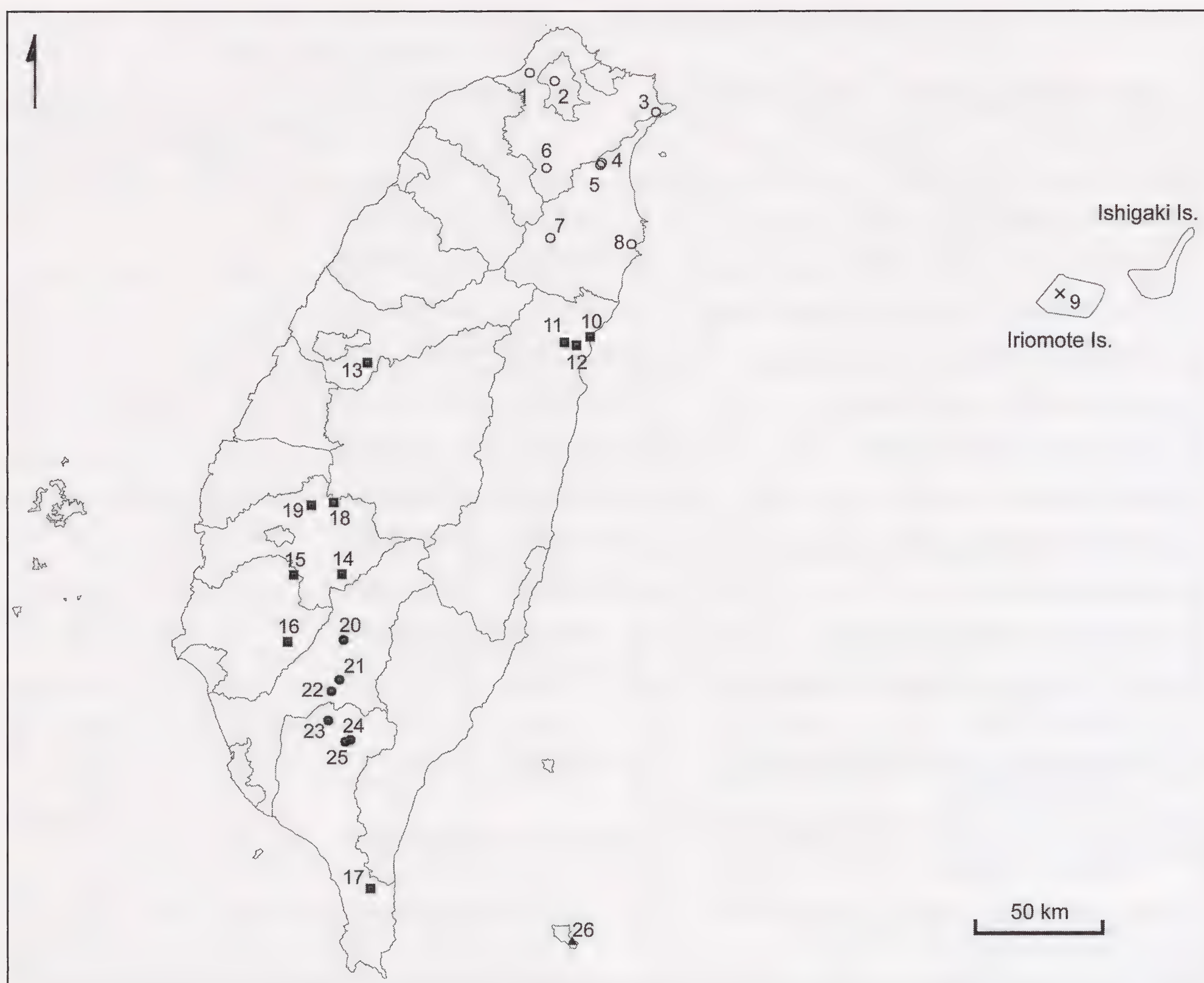


FIG. 1. Geographic range of the *Cyclotus* species complex. Different symbols illustrate the sampling sites of the five taxa. Open circle: *C. taivanus adamsi*; solid square: *C. t. dilatatus*; solid circle: *C. t. taivanus*; solid triangle: *C. t. diminutus*; X: *C. t. peraffinis*.

An approximately 860-bp fragment of the mitochondrial 16S rRNA gene was amplified by PCR using primers 16SRT (5'-ACA TAT CGC CCG TCA CTC TC-3') and 16SL900 (5'-AAA TGA TTA TGC TAC CTT TGC-3'), and an exactly 531-bp fragment of the COI gene was amplified using primers LCO1490 (5'-GGT CAA CAAATC ATAAAG ATATTG G-3') and HCO2198 (5'-TAA ACT TCA GGG TGA CCA AAA AAT CA-3') (Folmer et al., 1994). For COI some individuals got poor PCR products using primers LCO1490 and HCO2198. For those we use primer CoL90 (5'-TAG TGT TAAAT TAC GAT CAG T-3') and CoR600 (5'-AAG TCT TCT AAT TCG TGC AGA A-3'). The PCR reactions contained 10–50 ng/μl template DNA, 10 pmol of each primer, 5 μl 10× reaction buffer (10 mM Tris-HCl, pH 9.0, 50 mM KCl, 1.5 mM MgCl₂, 0.1% gelatin, 1% Triton X-100), 0.4 μl 25 mM/μl dNTP, 0.2 μl 50 mM

Mg²⁺, and 0.4 μl Taq polymerase (5 unit/μl) in a total volume of 50 μl. Thermal cycling for 16S rRNA was performed with an initial denaturation for 5 min at 95°C, followed by 30 cycles of 30 s at 95°C, 45 s at 57°C, 50 s at 72°C and final extension at 72°C for 10 min, final holding at 4°C. Thermal cycling for COI was performed similarly, with the exception that the annealing temperature was changed to 47°C. The PCR products were then purified using a purification kit (AMP PCR purification, Beckman) and then sequenced using an ABI 3700 autosequencer.

Phylogenetic Analyses

The COI + 16S rRNA data of *Cyclotus taivanus* ssp. were combined with data of the outgroup *Ptychopoma wilsoni* (Pfeiffer, 1865), *Cyclophorus moellendorffi* Schmacker & Boettger, 1891,

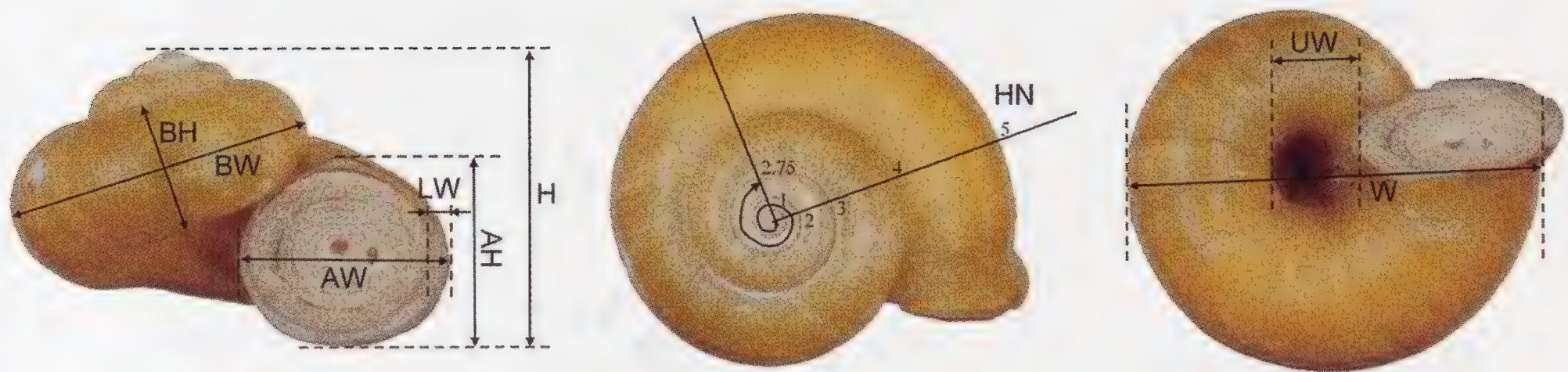


FIG. 2. The morphological characters of *Cyclopus*. AH = aperture height, AW = aperture width, BH = body whorl, BW = body whorl width, H = height, HN = whorl number, LW = outer lip width, UW = umbilicus width and W = width.

and *Cyclophorus friesianus* Moellendorff, 1883 (GenBank accession number No. HM753719, HM753951, HM753893, HM754125). We choose these as outgroup because *Ptychopoma* is a sister group of *Cyclopus* (Lee et al., 2008). However, the use of a closely related sister group as outgroup may not always lead to the correct phylogenetic tree (Lyons-Weiler et al., 1998; Sanderson & Shaffer, 2002), and it can be solved by using multiple outgroups. We use *Cyclophorus* that is outside of, but closely related to the ingroup as additional outgroup. Sequences were assembled and edited using Bioedit 5.0.9 (Hall, 1999). All alignments were performed using Clustal X (Thompson et al., 1997) and were manually proofread.

All data sets were subjected to maximum likelihood (ML) analyses using PhyML 3.0 (Guindon & Gascuel, 2003) and to neighbor-joining (NJ) analysis using MEGA 4.0 (Tamura et al., 2007). The substitution model used for the data set corresponded to the TVM+I+G model. It was the best models found using jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008). Before model fitting, the full-length sequences were tested to confirm that there was no significant heterogeneity in base frequencies across taxa (in COI: $X^2 = 10.91$, $df = 108$, $P = 1$; in 16S rRNA: $X^2 = 37.17$, $df = 132$, $P = 1$). NJ bootstraps consisted of 1000 iterations. The reliability of the ML trees was estimated by the approximate likelihood ratio test (aLRT) (using a custom-defined model, base frequencies: A = 0.3488, C = 0.1561, G = 0.1150, T = 0.3802) using PhyML 3.0. Gaps (from insertions/deletions) were treated as missing data.

Neutrality was tested using Tajima's D (COI: $D = -0.53034$, $P > 0.10$; 16S rRNA: $D = -0.75349$, $P > 0.10$) and Fu & Li's F statistic (COI: $F = 0.23658$, $P > 0.10$; 16S rRNA: $F = 0.26132$, P

> 0.10) (Fu & Li, 1993; Tajima, 1989). There were no selective effects on COI and the 16S rRNA gene.

Associations of the genetic, morphological and geographic distance between populations were explored through Mantel tests in XLSTAT (2010) on the pairwise genetic p-distance, pairwise similarity of shell characters (using Euclidean distance calculated by Primer 5.1.2 (2000)), and pairwise geographic distance. On the pairwise population fixation indices derived from AMOVA analysis (using Arlequin 3.5) (Excoffier & Lischer, 2010).

RESULTS

Morphological Analysis

The first three principal components accounted for 49.59%, 22.38% and 9.68% of the total variation in the matrix. The loadings of characters on the principal components reflected distinct groups of shell traits: umbilicus proportion (PC1), outer lip proportion (PC2), and aperture proportion (PC3). The *C. adamsi* group (sampling sites 1–8) was fully separated from the other four taxa (sampling sites 9–27) along the two axes, but *C. peraffinis*, *C. dilatatus*, *C. taivanus* and *C. diminutus* partially overlapped (Fig. 3).

Phylogenetic Analysis

Alignment of the COI + 16S rRNA datasets yielded 1,375 characters, of which 544 were variable sites. Of these 544 positions, 481 (88.42%) were parsimony informative. The average p-distance among haplotypes was 0.078. Sequence divergence among the haplotypes ranged from 0.001 to 0.126.

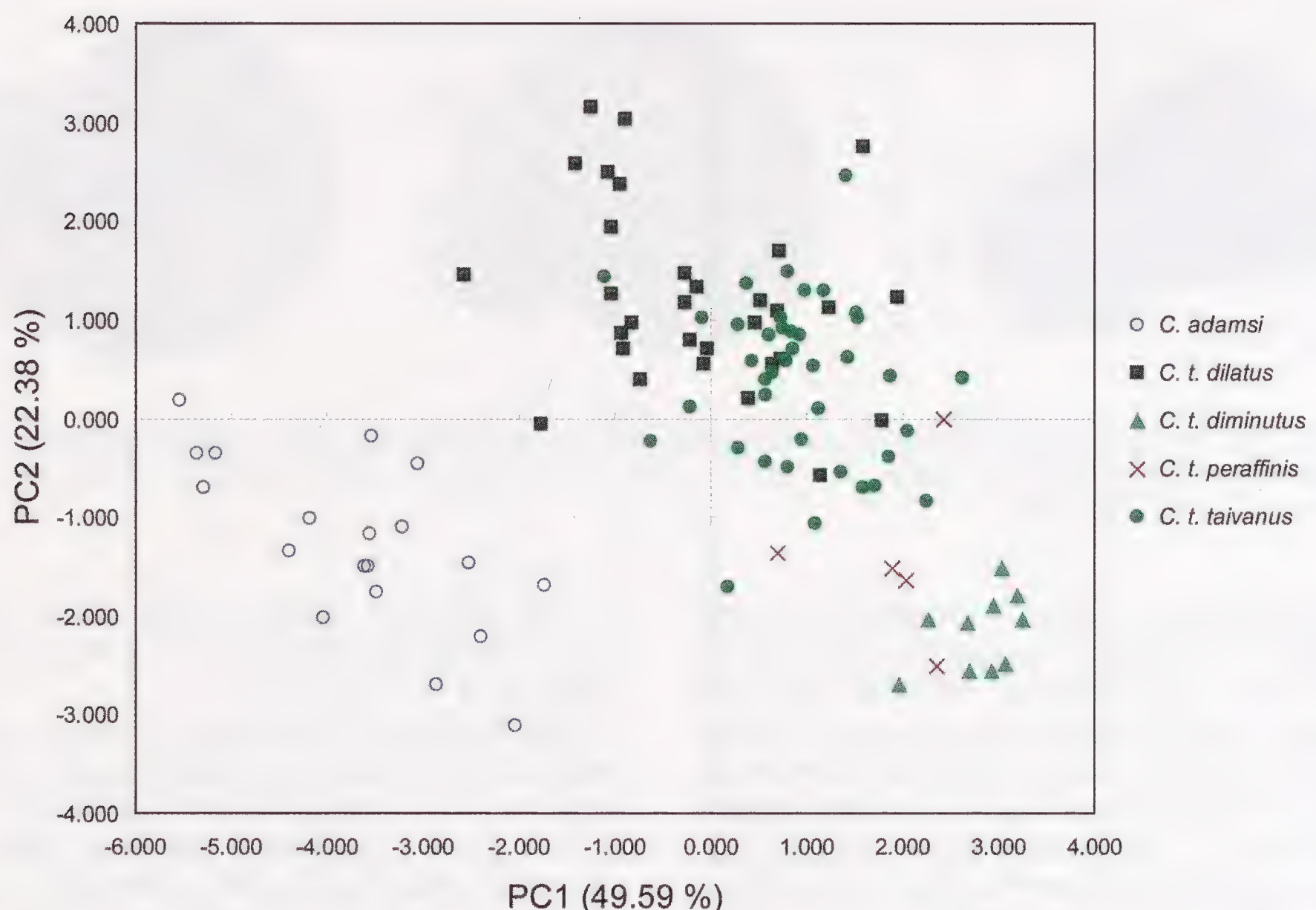


FIG. 3. Scatter plot of PCA scores on the first two axes of principal component analysis of *Cyclotus* shell morphological variables. Axis 1 accounts for 49.59% and axis 2 for 22.38% of the total variance. Different symbols illustrate the sampling sites of the five taxa, as described in Fig. 1.

The COI + 16S rRNA cladograms constructed using different tree-building methods exhibited a similar topology, differing only minimally on the terminal branches; therefore, we present only the ML tree here (Fig. 4). The topology of the COI + 16S rRNA gene tree strongly suggested the presence of two fundamental haplotype clades named A and B. Analyzing each clade separately, the average sequence divergence between haplotype clades A and B was 0.119 changes per site, which is relatively high compared with the changes within clades A and B (0.01 and 0.051, respectively). *C. adamsi* was clustered in clade A, while clade B comprised *C. peraffinis*, *C. dilatus*, *C. taivanus*, and *C. diminutus*.

The very high F_{ST} s among clade A and clade B, derived from AMOVA analysis indicated the absence of gene flow between these two clades. Beside, the high F_{ST} indicated limited gene flow between them.

Environmental Characterization of Sites

A PCA scatter plot of the sampling sites shows that the sampled populations primarily

comprise (axis 1, 44.03% of total variation) an environmental gradient from north Taiwan, with a low temperature, to south Taiwan, with a high temperature (Fig. 5). The second most important (axis 2, 32.42%) is a gradient with a more changeable temperature (annual range of monthly normal temperature), lower warm-season precipitation, and higher cold-season precipitation from north to south Taiwan.

When the results of molecular species characterization are superposed on the PCA scatter plot (Fig. 5), it becomes clear that clade A populations are mainly situated in the climate region with the variable temperature, lower warm-season precipitation and higher cold-season precipitation.

Mantel Test

There are four subspecies with variable shell morphology in clade B. Therefore, we performed the Mantel test to investigate the correlation between molecular data, morphology and geographic distribution.

The Mantel test to examine the association between the geographic distance separating

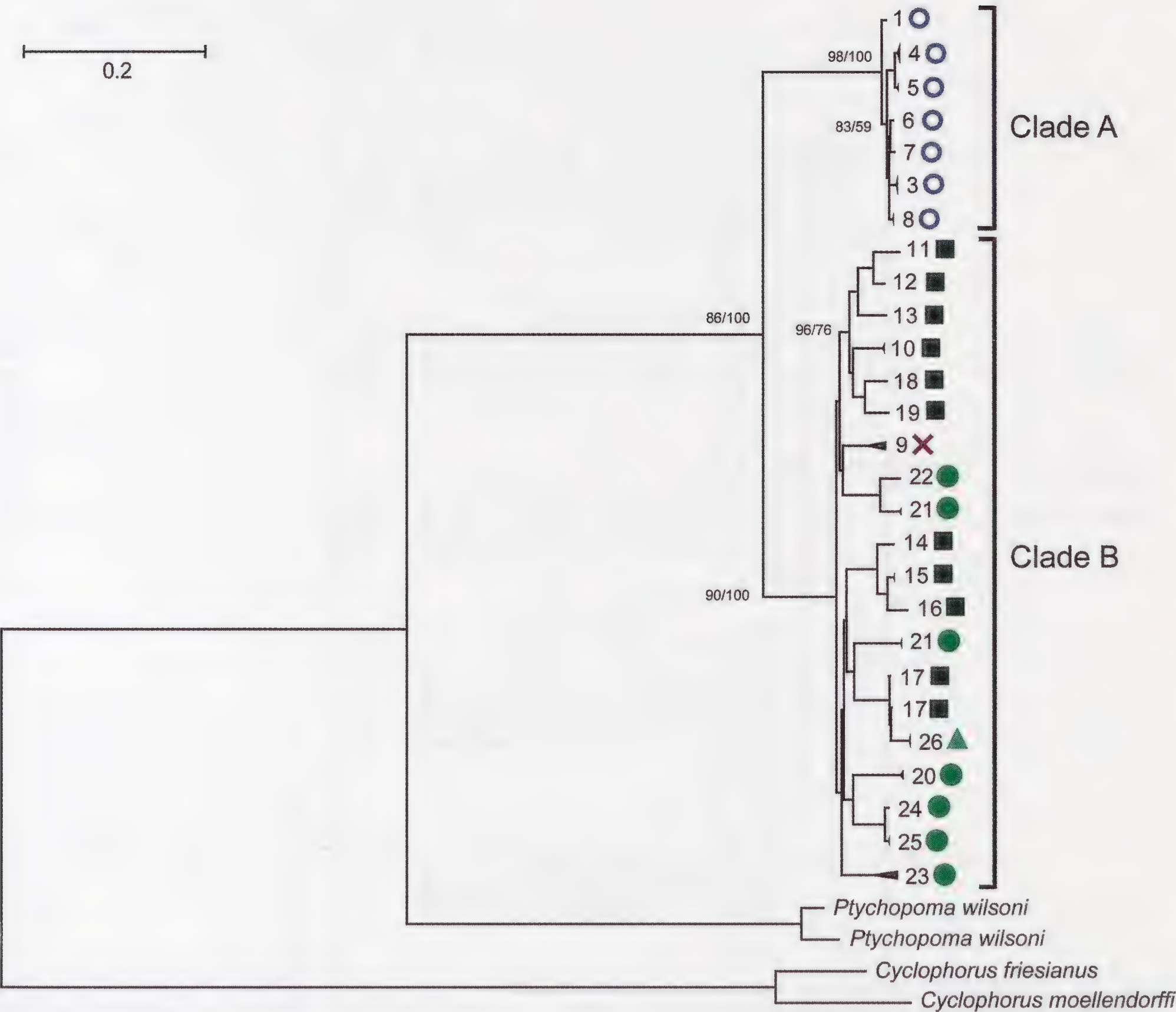


FIG. 4. Molecular phylogeny of *Cyclotus* based on concatenated COI and 16S rRNA gene sequences, with aLRT score / NJ bootstrap node. Branch tips are labeled with the sampling site numbers and the five taxa. Open circle: *C. adamsi*; solid square: *C. t. dilatus*; solid circle: *C. t. taiwanus*; solid triangle: *C. t. diminutus*; X: *C. t. peraffinis*. Some detail branches are compressed in terminal triangles.

populations and the molecular pairwise p-distance was significant (COI + 16S p-distance / geographic distance Mantel test, $r = 0.253$, $P < 0.0001$). In contrast, there were no correlations between the molecular pairwise p-distance and overall morphological similarity (COI + 16S p-distance / morphological similarity Mantel test, $r = 0.038$, $P = 0.436$). The Mantel test was also performed between the pairwise geographic distance and the overall morphological similarity matrix, and a significant correlation between them was observed ($r = 0.368$, $P < 0.0001$).

PLS Analysis

PLS analysis detected a significant correlation between shell traits and environmental

variables of clade B for the respective sampling sites (Table 2). We found a negative correlation between lower cold-season precipitation and outer lip breadth, a positive correlation between lower cold-season precipitation and degree of whorl increase, and a positive correlation between lower cold-season precipitation and whorl number.

DISCUSSION

Two Evolutionary Lineages in *C. taiwanus* ssp.

Morphologic and genetic variation within and among populations was detected in the present survey over the range of the taxon described as

TABLE 2. Correlations between shell traits and environmental variables in *C. taivanus* group (clade B in Fig. 4).

Shell trait	Correlation						
	Warm season mean tem- perature	Cold season mean tem- perature	Annual mean temperature	Annual range of monthly normal temperature	Warm season amount of precipitation	Cold season amount of precipitation	Annual amount of precipitation
size (H × W)	-0.032	-0.337	-0.198	0.161	0.280	-0.527	-0.388
tallness/flatness (H/W)	0.026	-0.003	0.005	0.037	0.070*	-0.081*	-0.053*
aperture proportion (AW/W)	-0.255	-0.454	-0.399	0.118**	0.189**	-0.420*	-0.359**
aperture shape (AW/AH)	0.247	0.422	0.368	0.084	-0.316	0.463	0.252
umbilicus proportion (UW/W)	0.337	0.423	0.423	0.045	-0.218	0.398	0.275
outer lip proportion (LW/AW)	-0.406	-0.599	-0.569	0.061**	0.265**	-0.598**	-0.512**
flatness degree of body whorl (BH/BW)	0.025	-0.130*	-0.059*	-0.102	0.215	-0.212*	-0.093*
degree of whorl increase ([W-UW-LW]/HN)	-0.039	-0.307	-0.185	0.085	0.334*	-0.487**	-0.227**
whorl number (HN)	-0.011**	-0.373**	-0.203*	0.234**	0.150**	-0.514**	-0.579**

*Indicates a significant correlation at the 5% level; **Indicates a significant correlation at the 1% level.
Warm season: May, June, July, August, September.
Cold season: November, December, January, February, March.

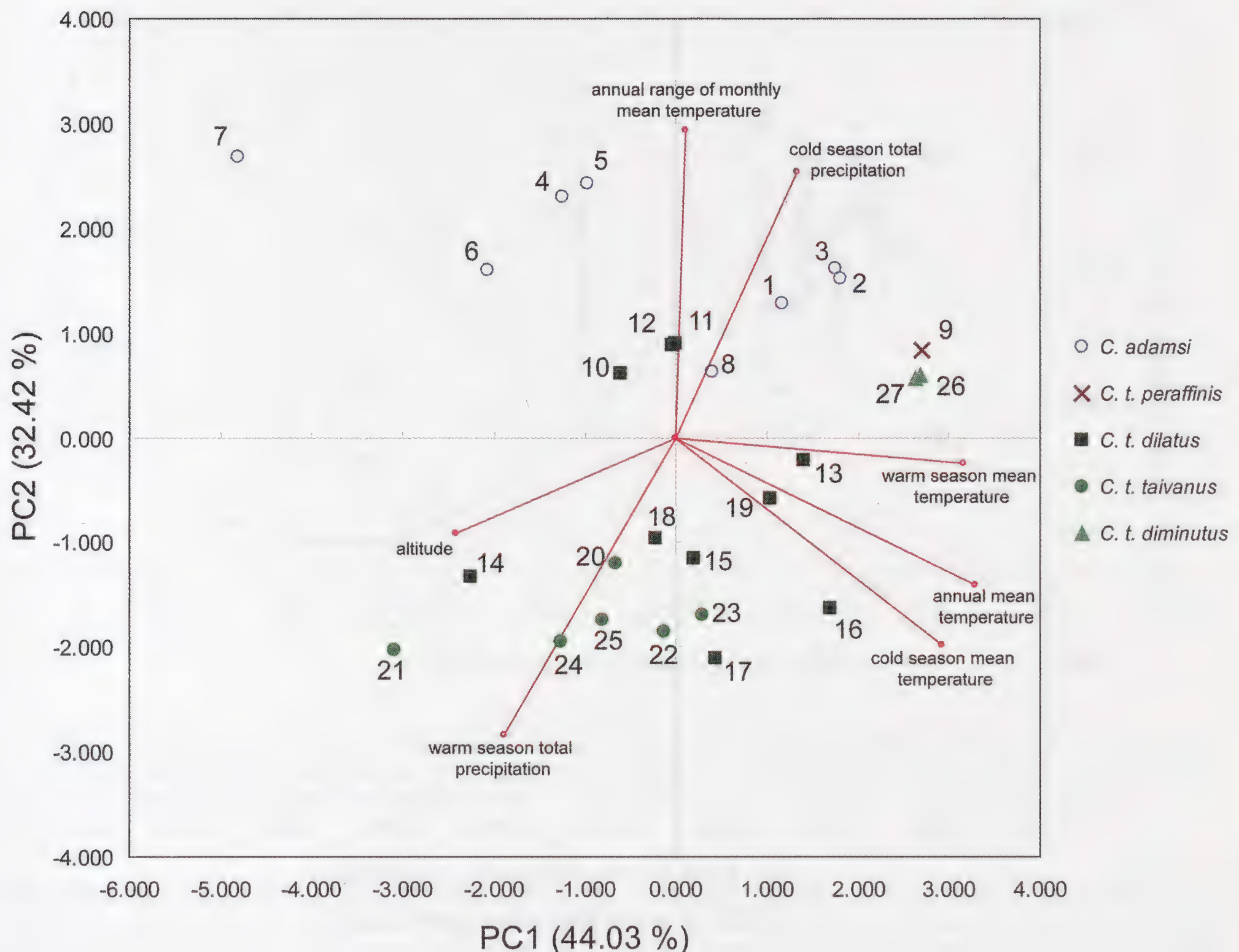


FIG. 5. Scatter plot of PCA scores on the first two axes of principal component analysis for environmental variables for 27 sites of *Cyclotus*. The correlation of variables with PCA axes is indicated by red line vectors. Sites where clade A populations were found are indicated in blue, clade B populations are indicated in brown, and series green color. Axis 1 accounts for 44.03% and axis 2 for 32.42% of the total variance. Different symbols illustrate the sampling sites of the five taxa, as shown in Fig. 1.

C. taivanus ssp. PCA revealed a clear distinction distribution of individuals in morph-space (Fig. 4), there were two highly divergent COI + 16S rRNA clades. The sequence divergence between the two clades was almost as high as that between other Cyclophoridae species (COI: 0.124 in *C. taivanus* ssp., 0.098–0.149 in other Cyclophoridae species; 16S rRNA: 0.107 in *C. taivanus* ssp., 0.083–0.087 in other Cyclophoridae species (Lee et al., 2008)). The average sequence divergence in COI and 16S rRNA among clade A and B equaled 0.124 and 0.107 changes per site, respectively. Owing to the lack of a calibration point, we have to assume that the two clades diverged approximately 5.4–5.5 million years ago, if the *Cyclotus* species has a similar mutation rate to that of Taiwan *Cyclophorus* (unpublished data; 2.28% and 1.95% per million years in COI and 16S rRNA, respectively).

The presence of two highly-divergent genetic clades or evolutionary lineages is therefore in concordance with morphological differences. This led us to the conclusion that there are two separately evolving lineages in *C. taivanus* ssp. This view is strengthened by the observation that clade A, found only in sampling sites 1–8 from north Taiwan (Fig. 1). The absence of gene flow between clade A and clade B suggested reproductive isolation of the clades (Table 3). Most species concepts, reviewed in Wilkins (2009, 2011) would recognize clade A as a separate species in the light of the presented evidence. Among these contemporary species concepts, unified species concept of De Queiroz (2007) is the best concept to apply in our case. This species concept comprises the biological species concept without proving actual reproductive isolation, and equates species with separately

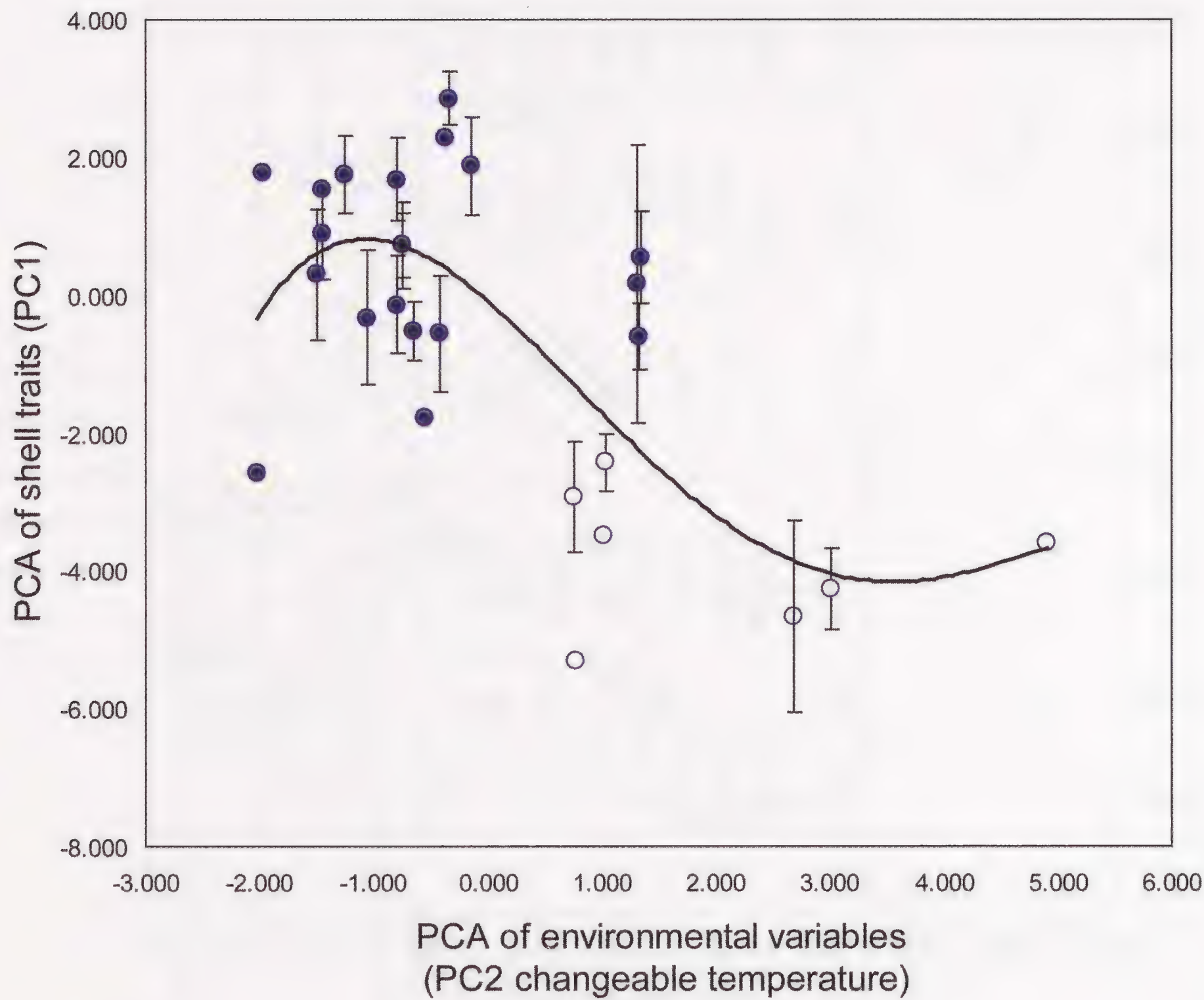


FIG. 6. Scatter plot of the environmental variables PC2 and shell traits PC1 averages ($\pm$ SD). Open and solid circles indicate clades A and B, respectively. The trend line is the 4th degree multinomial.

TABLE 3. Population pairwise F_{ST} s derived from AMOVA analysis of *Cyclotus* using COI and 16S rRNA data, respectively.

	<i>C. adamsi</i>	<i>C. t. dilatus</i>	<i>C. t. peraffinis</i>	<i>C. t. taivanus</i>	<i>C. t. diminutus</i>
COI					
<i>C. adamsi</i> (clade A)	0.00000				
<i>C. t. dilatus</i> (clade B)	0.71136*	0.00000			
<i>C. t. peraffinis</i> (clade B)	0.87074*	0.22362	0.00000		
<i>C. t. taivanus</i> (clade B)	0.69307*	0.19160*	0.22257*	0.00000	
<i>C. t. diminutus</i> (clade B)	0.95352*	0.44126*	0.78815*	0.39114*	0.00000
16S rRNA					
<i>C. adamsi</i> (clade A)	0.00000				
<i>C. t. dilatus</i> (clade B)	0.78897*	0.00000			
<i>C. t. peraffinis</i> (clade B)	0.92744*	0.40061*	0.00000		
<i>C. t. taivanus</i> (clade B)	0.81128*	0.24100*	0.29710*	0.00000	
<i>C. t. diminutus</i> (clade B)	0.94225*	0.48908*	0.98652*	0.48698*	0.00000

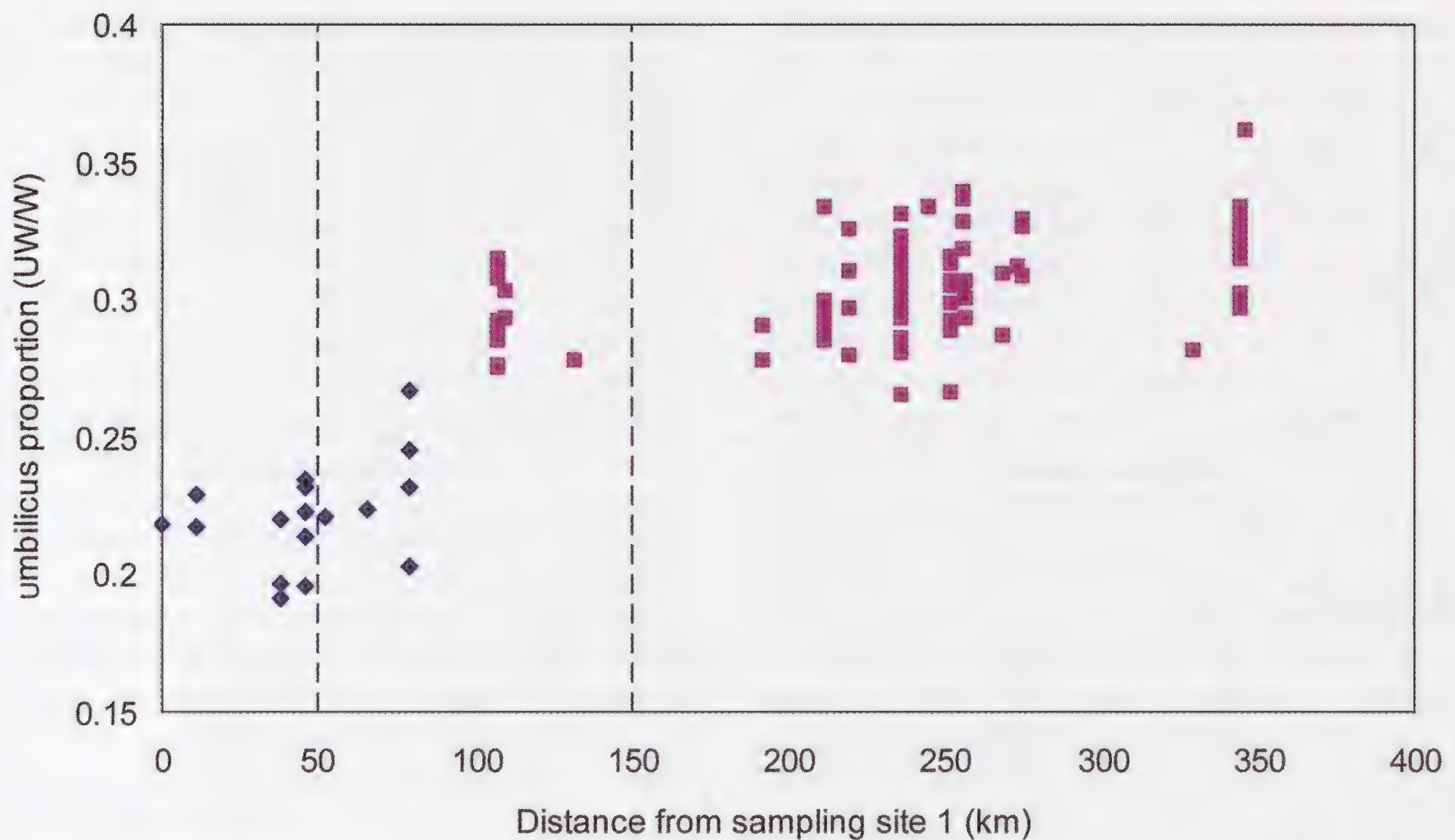


FIG. 7. The change in the umbilicus proportion of *C. adamsi* (blue rhombus) and the *C. taivanus* group (pink square) along the north to south population. Spots between dotted lines are measured from individuals around the contact zone.

evolving metapopulation lineages, which is appropriate in a case of allopatric relations. We recognized these two lineages (clade A and B) as different species according to the unified species concept, because the two lineages were reciprocally monophyletic and deeply divergent and showed a non-overlapping allopatric distribution, which indicates absence of gene-flow in these two lineages. For ease of description, and by comparing genetic clade and shell trait PCA data, we will refer to all individuals with the haplotype of clade A as *C. adamsi* (a valid species) and those with the haplotype of clade B as the *C. taivanus* group (*C. t. dilatatus*, *C. t. diminutus*, *C. t. peraffinis*, and *C. t. taivanus*) hereafter.

Portions of the molecular and phenotypic variation were shown to be due to an obviously long separation of *C. adamsi* and the *C. taivanus* group. An initial survey of the geographical distribution of these two taxa indicated that they show no differences in habitat preferences. *Cyclotus adamsi* and the *C. taivanus* group are never sympatric. In the scatter plot of environmental variables PC2 and shell traits PC1, *C. adamsi* appears to have adapted well to an unstable, arid warm season, a moist cold season and a cold temperature, but the *C. taivanus* group has not (Fig. 6). Temperature may be a limiting factor in the distribution of *C. adamsi* and the

C. taivanus group. Contrary to *C. adamsi*, the *C. taivanus* group with a flatter shell inhabits a warmer environment with a stable temperature. The warm and stable climate tends to have dense vegetation, and the flat shell is a supposed advantage such as less friction, when roaming over the ground in dense vegetation.

Hypothetical *C. taivanus* ssp. Speciation Model

It appears that a climatic gradient is responsible for the distribution patterns of the species (Fig. 5). Along this cline, *C. adamsi* occupies only sites with a moist winter and an arid summer climate. This type of climate appears to exclude the *C. taivanus* group. The actual level of water stress at a particular site depends strongly on the local microclimate, which may account for the observed intermingled pattern in the contact zone. Even though the sampling population was limited, there is probably a cline in terms of umbilicus proportion between populations near the contact zone (Fig. 7). Likewise, there are more or less clines in the other eight shell traits between populations near the contact zone (Fig. 8). Even though premating or postmating reproductive isolation may have evolved as a byproduct of ecological divergence, the ecological divergence appears

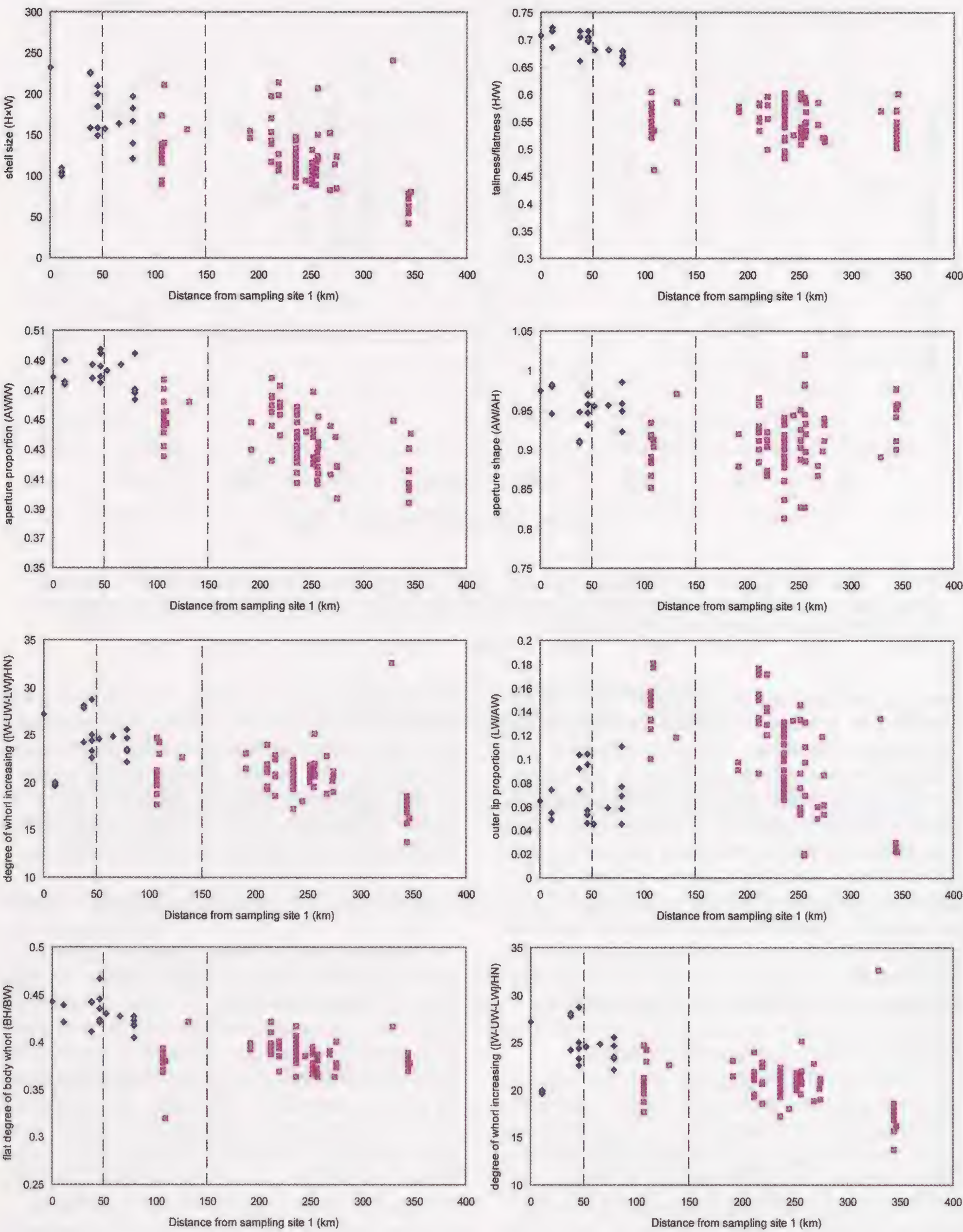


FIG. 8. The change in eight shell traits of *C. adamsi* (blue rhombus) and the *C. taivanus* group (pink square) along the north to south population. Spots between dotted lines are measured from individuals around the contact zone.

to be sufficient to prevent immediate contact, and therefore acts as an effective barrier to mating. The pattern of distribution along a climatic gradient suggests an ecotone divergence. Clines or ecotones are thought to promote the evolution of specialized adaptations in populations on either side of the border (Pfenninger et al., 2003). The speciation between *C. adamsi* and the *C. taivanus* group may be due to ecological speciation. An alternative hypothesis is that it is due to vicariance events and secondary contact. Parental individuals may never migrate between environments in the process of allopatric or parapatric speciation. However, ecological speciation may occur and the adaptations of local environment lead the evolution of reproductive isolation. The alternative model of speciation offers a much less likely scenario. There was no evidence to indicate an obstacle to dispersal around the contact zone in terms of historical geology.

Associations between Molecular and Morphological Variability within the *C. taivanus* Group

In the morphological PCA scatter plots of the *C. taivanus* group, all of its members partially overlap (Fig. 3). In particular, the shell morphology of *C. t. diminutus* and *C. t. peraffinis* are similar, and as expected their shell trait PCA scatter plots overlap (Fig. 3). However, there is limited gene flow between them (Table 3). The results of the Mantel tests indicated that the null hypothesis of no association between molecular p-distance and overall morphological similarity in shell traits could not be rejected within the *C. taivanus* group. This suggested that the observed shell trait differences have not evolved in concordance with the mitochondrial genome, and as a result the phenotypic population structure does not reflect the phylogenetic history of the populations.

Associations between the Environment and Morphological Variability within the *C. taivanus* Group

The results of PLS analysis showed that differences between populations in some traits covaried significantly with the long-term climatic conditions of the sampling site. We found that precipitation is correlated with some shell traits of the *C. taivanus* group. Cold-season precipitation and outer lip proportion are significantly negatively correlated; in contrast, warm-season precipitation and outer lip proportion are significantly positively correlated.

The same applied to whorl number, sampling sites with a greater annual precipitation which are associated with dry summer in Taiwan (Lin, 1990), being associated with a lower whorl number. This appeared astonishing at first, because a humid climate allows for greater feeding activity, and snails should therefore attain a greater number of whorls. A similar tendency towards smaller snails occurring in habitats with more rainfall was observed by Goodfriend (1986), and can possibly be explained as an adaptation of sexual maturity. There is no further whorl growth and forming an aperture lip when *Cyclotus* reaches sexual maturity. Reproducing earlier in a wetter cold season would offer the next generation the possibility of reaching a larger size before summer. Early reproduction could thus constitute a selective advantage, because a small size of juveniles of most snails results in high mortality in the dry summer of Taiwan (experience from field work). The ecological divergence probably appears rule of speciation in the case of *C. taivanus* ssp.. Nevertheless, the speciation process is not complete among *C. t. dilatatus*, *C. t. diminutus*, *C. t. peraffinis*, and *C. t. taivanus*, and the continued adaptation to climatic pressure is a rule of the speciation process. However, further experiments are needed to determine whether the size/precipitation correlation in the *C. taivanus* group is due to phenotypic plasticity in response to the prevailing conditions or whether it has an adaptational significance.

In conclusion, we consider the phenotypic and phylogenetic divergence between the two identified lineages sufficiently large to propose the presence of two distinct evolutionary entities as valid species (*C. adamsi* and the *C. taivanus* group). The speciation process between *C. adamsi* and the *C. taivanus* group is complete, as evidenced by a lack of gene flow (Table 3). The reproductive isolation of the two taxa may be due to divergent selection in traits in different environments. In other words, the speciation model in this case is ecological speciation. However, more sampling sites around the contact zone should be contained in further studies. For the subordinate members of *C. taivanus*, there is no resolution in phylogeny analysis of the present investigation. To resolve the relationships in the *C. taivanus* group, more rapid evolved molecular marker is needed for further study. In addition, hypotheses regarding correlations between *C. taivanus* group divergence and environmental variables could be tested in future studies.

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ANANCYLUS ROSANAE (GASTROPODA: PULMONATA), NEW GENUS AND NEW SPECIES, FROM IGUAZÚ NATIONAL PARK, ARGENTINA

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ABSTRACT

Seventeen species of ancyliids occur in southern South America, seven in Argentina. ***Anancylus rosanae***, n. gen. et sp., is described based on characters of the shell, radula, jaw and reproductive system. This new genus, found in the upper Iguazú River rapids, Iguazú National Park, Misiones Province, Argentina, has the following distinctive features: patelliform shell, usually low, with a circular apex located at the anterior end of the shell; three adductor muscles: large posterior (twice the length of the anterior muscles) and two tear-shaped anterior; adhesive epithelium between the two anterior muscular insertions; no difference between the plates of the jaw.

Key words: ***Anancylus rosanae***, n. gen. et sp., Ancyliidae, shell, anatomy, Argentina.

INTRODUCTION

American patelliform freshwater gastropods have been included within Ancyliidae, Acroloxiidae and Lymnaeidae. Only the Ancyliidae are present in South America. In southern South America (Brazil, Argentina, Uruguay, Paraguay and Chile), this family is represented by *Anisancylus* Pilsbry, 1924 (2 species), *Burnupia* Walker, 1912 (1 species), *Ferrisia* Walker, 1903 (1 species), *Gundlachia* Pfeiffer, 1849 (6 species), *Hebetancylus* Pilsbry, 1913 (2 species), *Laevapex* Walker, 1903 (1 species) and *Uncancylus* Pilsbry, 1913 (4 species) (Lanzer, 1996; Santos, 2003; Simone, 2006). The following species have been recorded in Argentina: *Uncancylus concentricus* (d'Orbigny, 1835), *Hebetancylus moricandi* (d'Orbigny, 1837), *Anisancylus obliquus* (Broderip & Sowerby, 1832), *Gundlachia ticaga* (Marcus & Marcus, 1962) and *G. radiata* (Guilding, 1828) (Fernández, 1981; Rumi et al., 2006, 2008; Ovando et al., 2011). Of the species found in Argentina, the first two have a widespread distribution, whereas *A. obliquus* has only been recorded in the south and centre, and the other two species – *G. ticaga* and *G. radiata* – have been recorded only in the provinces of Misiones and Jujuy, respectively (Gutiérrez Gregoric et al., 2006; Rumi et al., 2006, 2008; Ovando et al., 2011). Recently, *Ferrisia* was reported from

northwest Argentina and *Laevapex* was cited from the northeast (Iguazú River) (Gutiérrez Gregoric et al., 2006; Rumi et al., 2006, 2008; Agudo, 2007; Ovando & Santos, 2011).

The taxonomic placement of the Ancyliidae has changed over the years. This freshwater limpet clade has been recognized at the family level as the Ancyliidae (Boss, 1982; Brown, 1994; Santos, 2003; Albrecht et al., 2004; Simone, 2006; Walther, 2008; Ovando et al., 2011); alternatively, it has been placed within the larger family Ancyloplanorbidae (= Planorboidea) (Hubendick, 1978); and it has also appeared as a subfamily or a tribe of Planorbidae (Burch, 1962; Starobogatov, 1967; Bouchet & Rocroi, 2005; Albrecht et al., 2006, 2007; Walther et al., 2010). Albrecht et al. (2004), based on molecular studies, included various genera in the Ancyliidae, but separated the genus *Burnupia* from the group. Later, Albrecht et al. (2006, 2007) proposed a new family (unnamed) for *Burnupia*. In the absence of a definite position, I will consider the genera previously mentioned of patelliform freshwater gastropods of Argentina as Ancyliidae.

In this study, the material from the Iguazú River, Misiones Province, Argentina, previously identified as *Laevapex* sp., was revised based on the shell and soft-body parts, and a new genus and new species, ***Anancylus rosanae***, were generated.

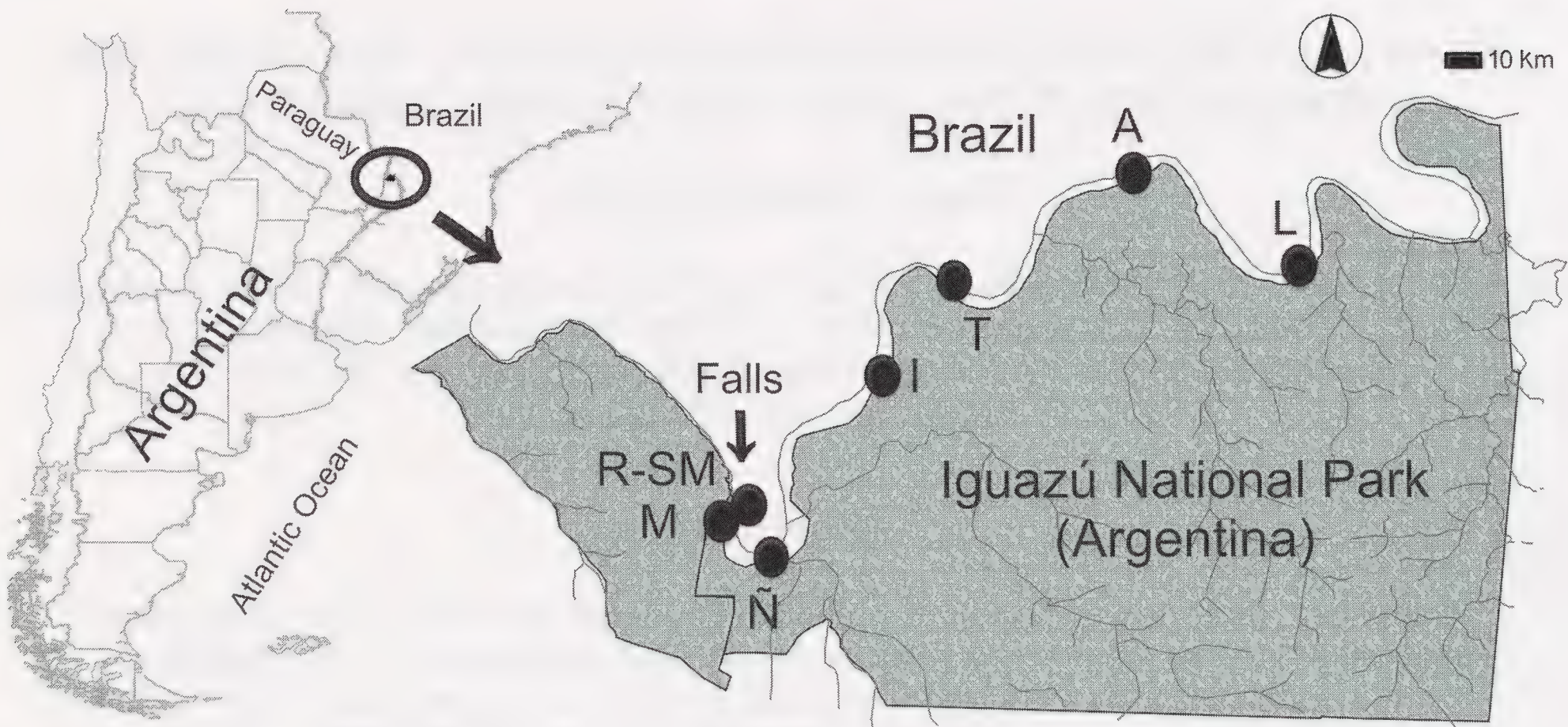


FIG. 1. Rapids of the Iguazú River where *Anancylus rosanae* was recorded, Iguazú National Park, Argentina. A: Apepú; I: Irene; L: León; M: Mbigua; Ñ: Ñandú; R - SM: Rivadavia and San Martín; T: Tacuara.

MATERIALS AND METHODS

Specimens were collected in 2004 and 2005 in Iguazú National Park, Misiones Province, Argentina (Fig. 1, Table 1). Soft parts were separated from the shell after relaxation in 10% Nembutal solution for 4 h and then fixed in modified Raillet-Henry solution for freshwater animals (93% distilled water, 2% glacial acetic acid, 5% formaldehyde and 6 g/l sodium chloride solution). Radulae and jaws were separated from the buccal mass and then cleaned with sodium hypochlorite (Clorox).

Five shell measurements were taken: total length (TL), anterior width (at apex) (AW), total width (TW), height (H) and diameter of apex (DA) (Fig. 2). Radulae (n = 3) and jaws (n = 2) were observed under a scanning electron microscope JEOL 6360 at the Museo de La Plata (Facultad de Ciencias Naturales y Museo – Universidad Nacional de La Plata). Internal anatomy (n = 8) was analyzed using a Leica MZ6 stereoscopic binocular microscope with *camera lucida*. For the anatomical studies, the methodology proposed by Santos (2003) was followed.

TABLE 1. Number of specimens collected during different months at each rapids where *Anancylus rosanae* was recorded, Iguazú National Park, Argentina.

	Ñandú	San Martín	Tacuara	Mbigua	León	Apepú	Irene	Rivadavia
South	25°42'	25°41'	25°36'	25°41'	25°36'	25°33'	25°38'	25°41'
West	54°25'	54°27'	54°21'	54°26'	54°14'	54°17'	54°23'	54°26'
Feb-04	25							7
Jun-04			13					
Feb-05			25		30	20	3	
Jun-05		5		8				
Dec-05	8							

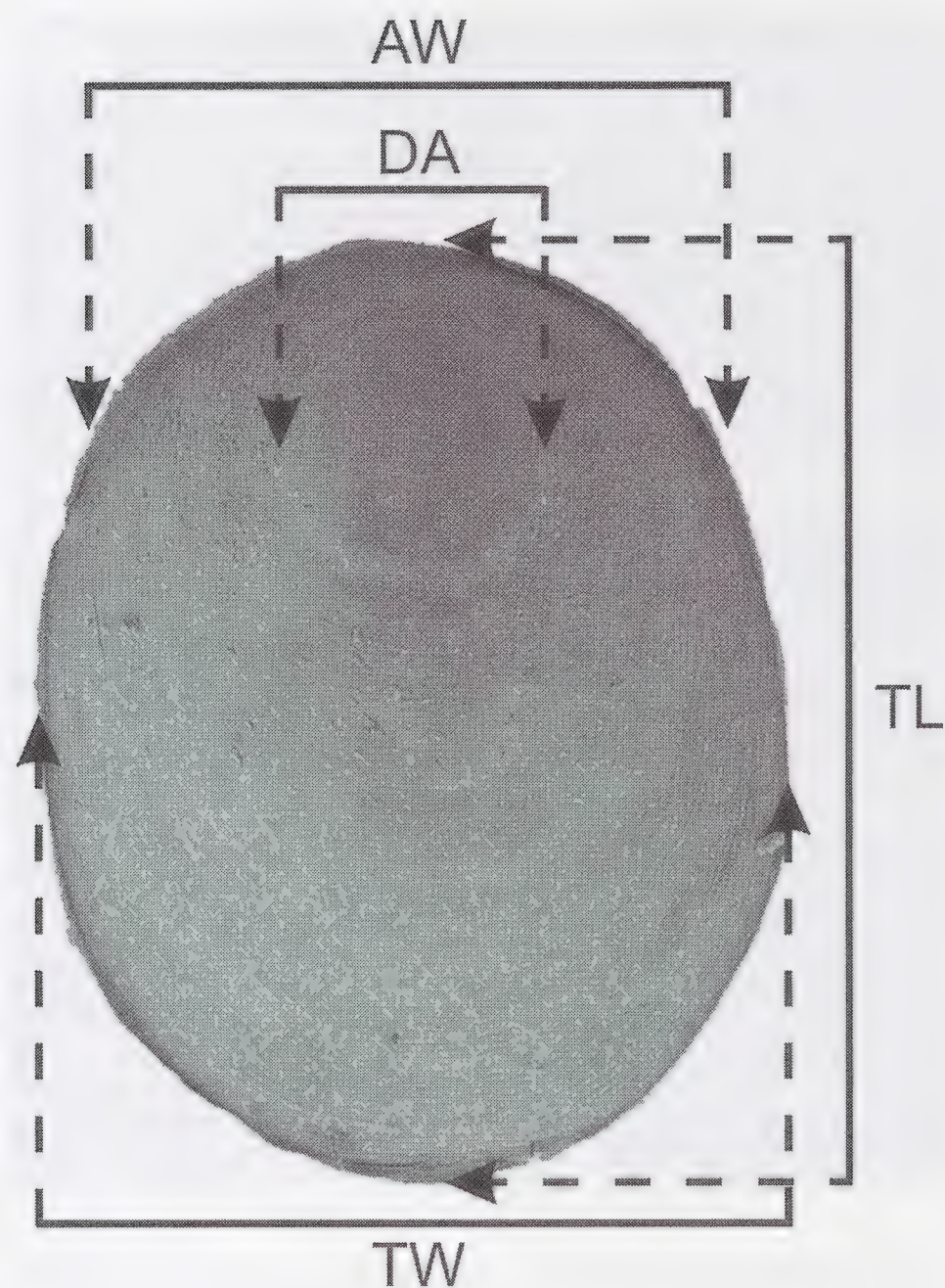


FIG. 2. Shell measurements used for *Anancylus rosanae*, n. sp.: TL: Total length; AW: Anterior width; TW: Total width; DA: Diameter of apex.

RESULTS

Anancylus gen. nov.

Type Species

Anancylus rosanae, sp. nov.; Argentina.

Diagnosis

Shell patelliform, usually low, circular apex located along midline at anterior end of shell. Three adductor muscles present: large posterior (twice length of anterior muscles) and two tear-shaped anterior muscles. Adhesive epithelium between two anterior muscles. Radula with more than one hundred rows of teeth. Central tooth asymmetric, tricuspid. First lateral tooth tricuspid, tall endocone. Jaw plates low and wide, undifferentiated. Free edge of plates denticulate. Genital system: tubular ovotestis, thick flagellum (three times longer than prepucium), tubular prolongation of uterus three times longer than ovotestis.

Etymology

An = without; *ancylus* = because of the hooked curved shape.

Differentiation

See Discussion below.

Anancylus rosanae sp. nov. (Figs. 2–18)

Laevapex sp.; Gutiérrez Gregoric et al., 2006: 51–60; Rumi et al., 2006: 199, 207; Agudo, 2007: 6; Rumi et al., 2008: 83, 102.

Type Locality

Upper Iguazú River, Iguazú National Park, Misiones Province, Argentina.

Type Material

Holotype (MLP: 13219); and paratypes (MLP: 13220, 7 specimens) housed at Museo de La Plata.

Etymology

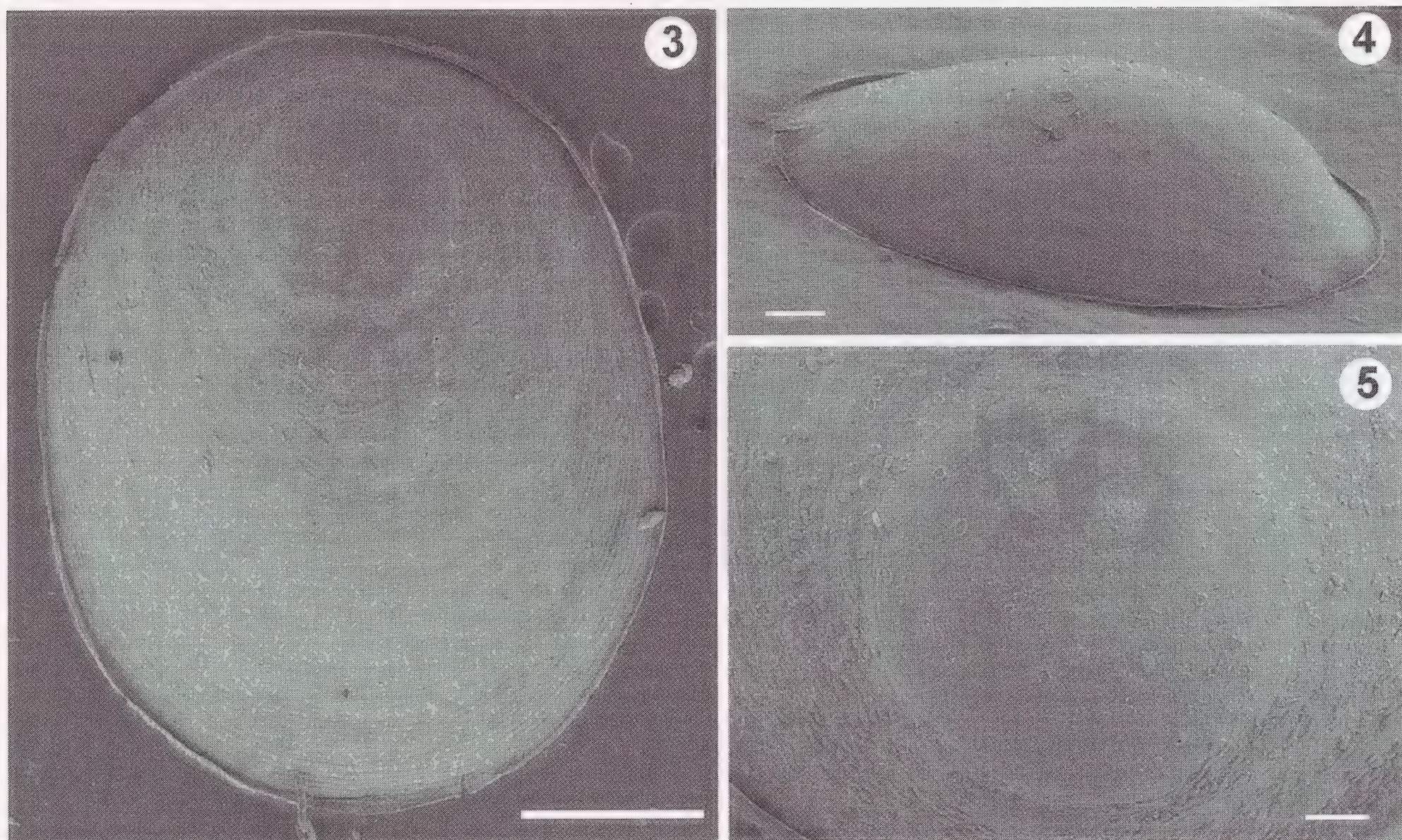
Dedicated to Rosane Lanzer.

Description

Shell (Figs. 2–7): Shell patelliform, small, low. Aperture elliptic, edge smooth. Anterior end narrower than posterior end. Periostracum hyaline (transparent), slightly yellow, fine concentric growth lines; no radial lines. Apex smooth, rounded, located along midline at anterior end of shell.

Measurements (in mm): TL: average 2.25 (N: 38; Range: 0.92 – 3.07; SD: 0.40); TW: average 1.74 (N: 38; Range: 0.77 – 2.42; SD: 0.33); AW: average 1.55 (N: 38; Range: 0.72 – 2.07; SD: 0.30); H: average 0.56 (N: 30; Range: 0.32 – 0.77; SD: 0.10); DA: 0.73 (N: 33; Range: 0.47 – 1.12; SD: 0.21). Table 2 shows measurements for the holotype and paratypes.

Body (Figs. 6, 8, 9): Tentacles cylindrical, not pigmented. Pigmentation of mantle concentrated in the center. Edges with radial bands diffusely pigmented. Three well-developed adductor muscles observed: two anterior and one posterior. Two anterior muscles tear-shaped,



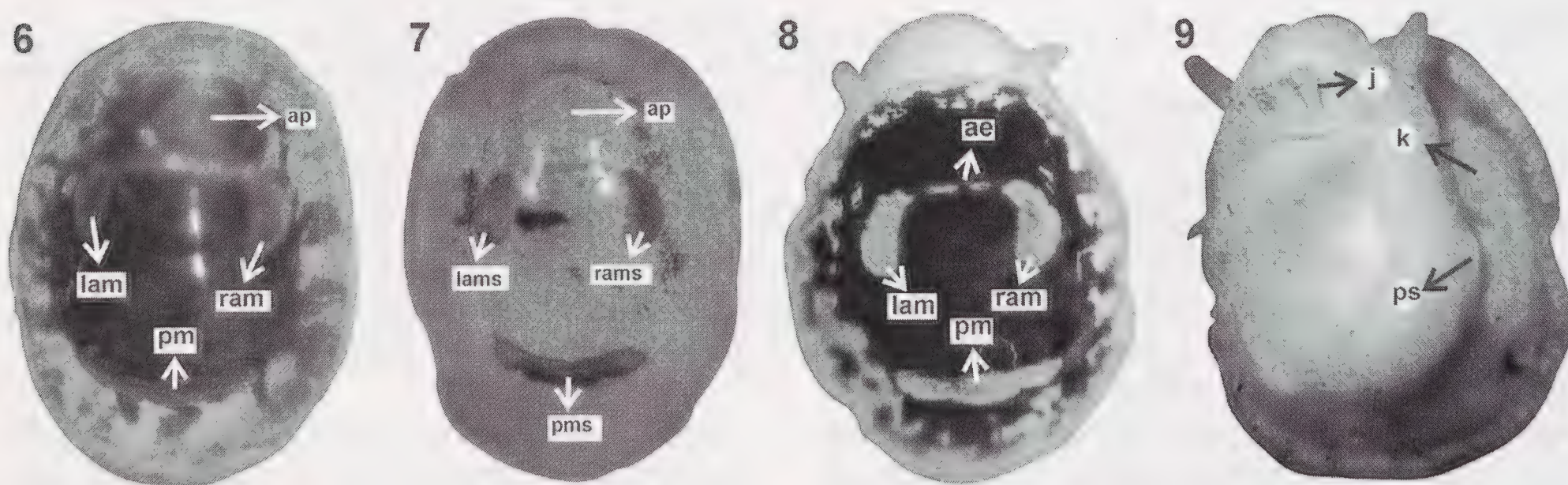
FIGS. 3–5. Shells of *Anancylus rosanae*, n. sp. FIG. 3: Dorsal view; FIG. 4: Lateral view; FIG. 5: Apex. Scale bars: FIG. 3 = 0.5 mm; FIG. 4 = 0.2 mm; FIG. 5 = 0.1 mm.

connected by adhesive epithelium (Fig. 8). Large posterior muscle (twice the length of anterior muscles), spans the full width of the visceral mass (46.6% of total shell width). Pseudobranch single-lobed, located behind female pore.

Digestive System: The digestive system has no features of special interest. Radular sac projects

beyond pharyngeal bulb into the visceral mass. Long intestine (two times the total body length), curved at the back, opens behind the pseudobranch.

Jaw (Figs. 10–13): Jaw horseshoe-shaped with 44 plates, all similar except for size. Plates rectangular, wider than tall. Free edge of plates with chitinous projections (Figs. 12, 13).



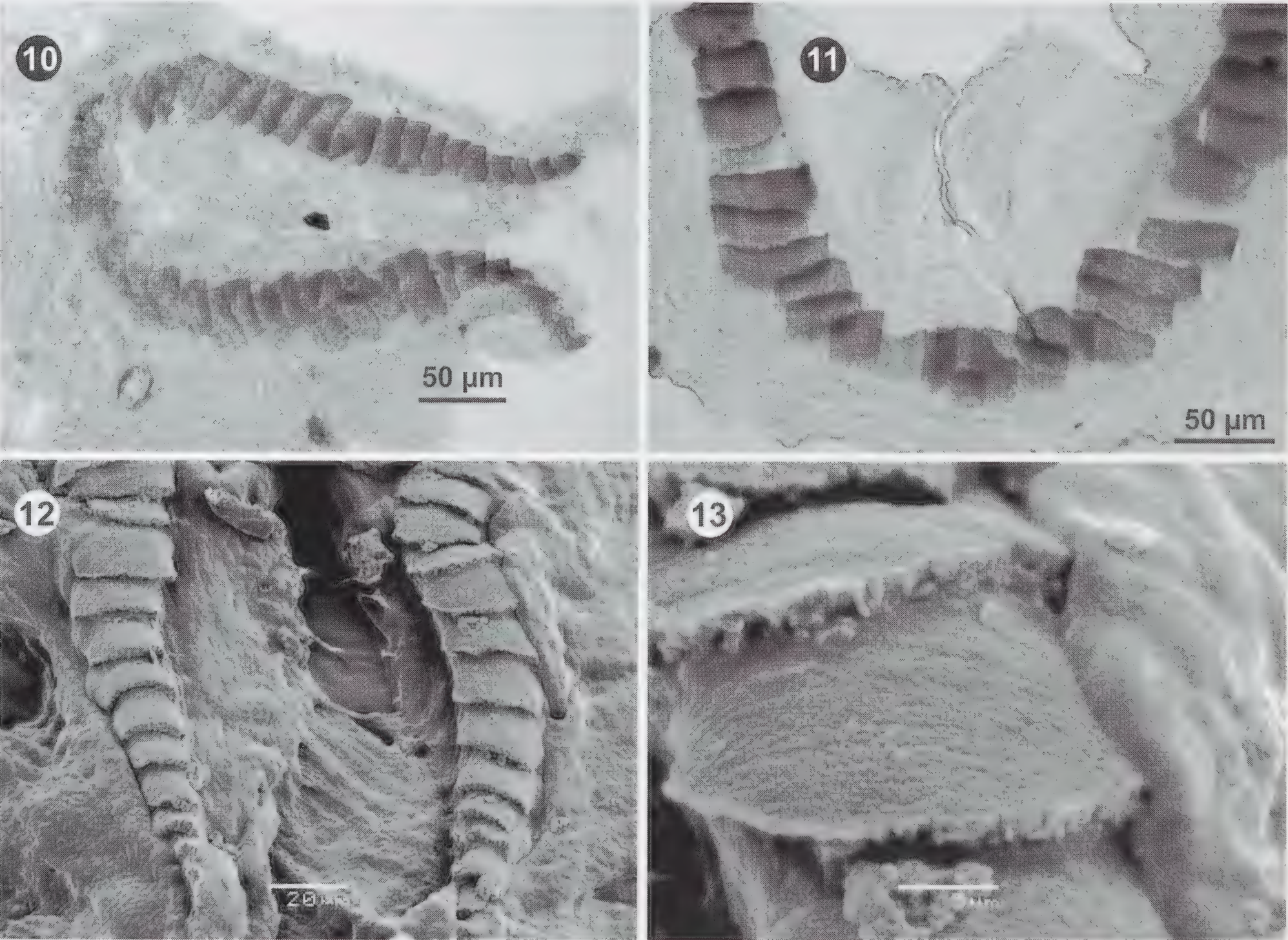
FIGS. 6–9. Shell and mantle of *Anancylus rosanae*, n. sp. FIG. 6: Translucent shell through which the mantle is observed; FIG. 7: Shell; FIG. 8: Mantle; FIG. 9: Ventral view of body. Abbreviations: ae: adhesive epithelium; ap: apex; j: jaw; k: kidney; lam: left anterior muscle; lams: left anterior muscle scar; pm: posterior muscle; pms: posterior muscle scar; ps: pseudobranch; ram: right anterior muscle; rams: right anterior muscle scar.

TABLE 2. Shell measurements (mm) of *Anancylus rosanae* (Holotype and Paratypes). TL: total length; AW: anterior width; TW: total width; H: height; DA: diameter of apex.

Site	Date	TL	TW	AW	H	DA
Ñandú rapids (Holotype MLP 13219)	Dec-05	3.08	2.43	2.08	0.75	0.68
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.25	1.88	1.63	0.55	0.60
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.43	1.73	1.33	0.73	0.60
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.50	1.90	1.70	0.65	0.60
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.65	2.05	1.80	0.63	0.65
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.70	2.05	1.78	0.68	0.58
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.73	2.20	2.03	0.55	0.65
Ñandú rapids (Paratype MLP 13220)	Dec-05	2.25	1.73	1.40	0.65	0.55

Radula (Figs. 14–17): Between 105 and 145 rows of teeth per radula (N = 3). Central tooth asymmetric, tricuspid, left cusp most developed. The base of the tooth – as well as the tooth itself – thin and uniform. First lateral tooth tricuspid, with endocone more developed. In

some teeth, a subcusp can be seen outside the ectocone. Base and body tooth thin. From tooth 18 on, subcuspids appear toward the inner side of endocone: one in teeth 18 and 19, two in teeth 20 and 21, and three between teeth 22 and the 23. After tooth 18, there is a reduction



FIGS. 10–13. Jaw of *Anancylus rosanae* n. sp. FIGS. 10, 11: General view and plates in optic microscope view; FIGS. 12, 13: Plates in scanning electron microscope view.

in tooth-size, ectocone and mesocone. The last teeth of the row (marginals) have seven cusps. The base of the tooth and its body widen from the central tooth towards the marginal teeth. Radular formula: $25 (\pm 2) - 1 - 25 (\pm 2)$.

Reproductive System (Fig. 18): Ovary tubular, embedded in the digestive gland. From the ovary emerges the hermaphroditic duct, which coils up to become the seminal vesicle and then continues up to the carrefour. Female and male reproductive elements are described separately.

Female Genital System: Uterus surrounded by nidamental and albumen glands (the former cannot be seen in Fig. 18). Large tubular prolongation of the uterus (three times the length of ovary). Small vagina that opens in front of the pseudobranch. Spermathecal duct begins near the female genital pore; duct tubular and of the same size as ovary.

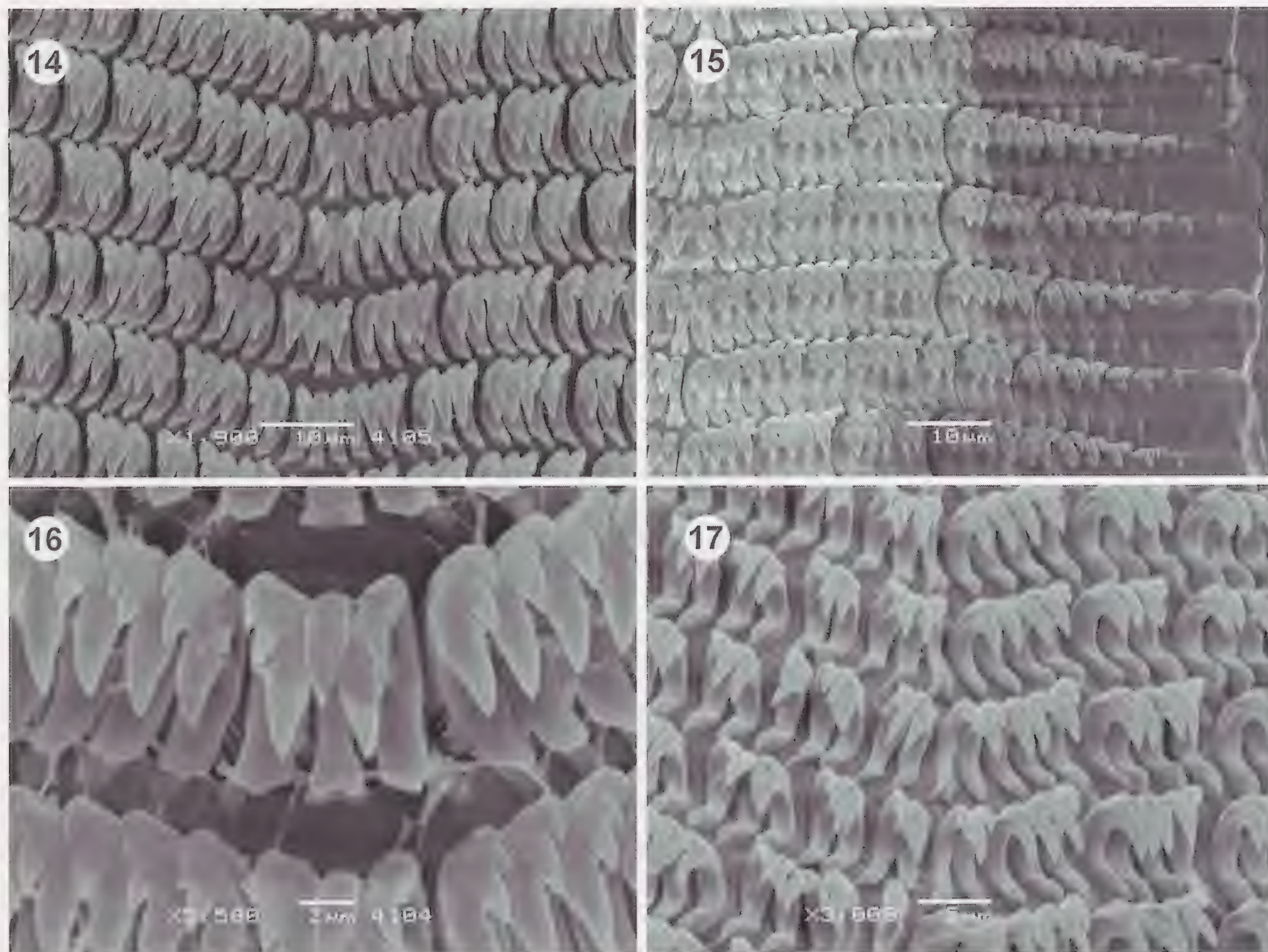
Male Genital System: Prostate emerging from the carrefour; tubular, smooth and thick. Vas deferens emerges from the prostate, crosses over the prepuce, and inserts at its proximal end. Flagellum large and thick (twice as thick as the vas deferens). Flagellum three times longer than prepuce. Male pore behind the left tentacle. Penis missing.

Distribution

The new species was found only at the type locality, that is, the rapids of the upper Iguazú River, Iguazú National Park (Table 1).

DISCUSSION

Previous identifications of *Anancylus rosanae* as *Laevapex* sp. for the upper Iguazú River (Gutiérrez Gregoric et al., 2006; Rumi et al., 2006, 2008; Agudo, 2007) may be explained by



FIGS. 14–17. Radula of *Anancylus rosanae*, n. sp. FIGS. 14, 15: General view; FIG. 16: Central tooth and first lateral teeth; FIG. 17: Posterior-dorsal view.

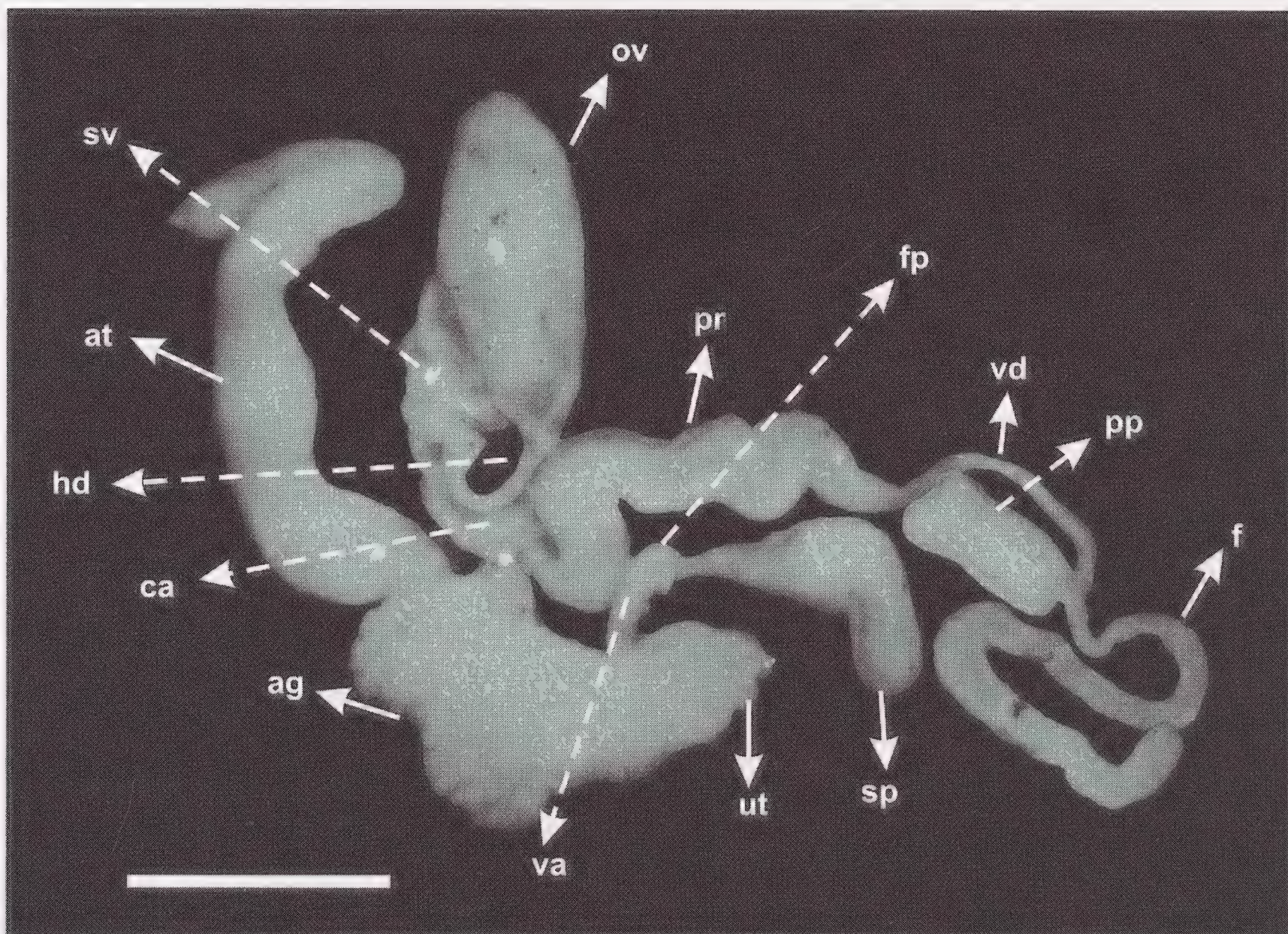


FIG. 18. Reproductive system of *Anancylus rosanae*, n. sp. Abbreviations: ag: albumen gland; at: tubular prolongation of uterus; ca: carrefour; vd: vas deferens; f: flagellum; fp: female pore; hd: hermaphrodite duct; ov: ovotestis; pp: prepuce; pr: prostate; sp: spermatheca; sv: seminal vesicle; ut: uterus; va: vagina. Scale bar: 0.5 mm.

the fact that its descriptive characters agreed with those published by Lanzer (1996) for *Laevapex*. Lanzer (1996) did not accompany the description with shell figures or anatomical characters. She mentioned that the apex of the shell is obtuse, smooth and posterior. In the species described herein, the apex is smooth and obtuse, but it is located along midline at the anterior end of the shell. Placement of this new species in *Laevapex* is not appropriate, because in this genus the apex is obtuse, just behind the middle of the shell and inclined to the right. Rumi et al. (2006, 2008) and Agudo (2007) took the information mentioned in Gutiérrez Gregoric et al. (2006) repeating the error with regard to placement of the apex. The anterior placement of the shell apex distinguishes *Anancylus* from other ancyliids, which until now were all described as having a central or posteriorly positioned apex.

Burch (1962) believed that the arrangement of adductor muscles is important in ancyliid systematics. He recognized three subfamilies: Ancyliinae, in which the adductor muscle is continuous and with a "C" shape; *Laevape-*ciinae has three small adductor muscles and

adhesive epithelium between the posterior and right anterior muscles; *Ferrissinae* with three small muscles and no adhesive epithelium. The muscle arrangement of *Anancylus rosanae* does not resemble that of any of these three subfamilies. It has two anterior tear-shaped muscles curved along their posterior side and connected by an adhesive epithelium; the posterior muscle is longer and not connected by adhesive epithelium with the anterior muscles. *Anancylus rosanae* shows a muscle arrangement and pigmentation of the mantle that is similar to that described by Lanzer (1996) for *Laevapex* sp. from southern Brazil. However, as it was mentioned, the location of the collected specimens in the Iguazú River, identified as *Laevapex* sp., was erroneous.

In *Anancylus rosanae*, the base and body of the radula's central tooth are thinner than in other members of the family Ancyliidae (*Anisancylus obliquus*, *Anisancylus dutrae* Santos, 1994, *Laevapex vazi* Santos, 1989, *Uncancylus concentricus*), although the general pattern is similar. *Anisancylus obliquus*, *A. dutrae*, *Gundlachia radiata* (Bourguignat, 1853), *Gundlachia ticaga*, *U. concentricus* and *Hebetancylus*

moricandi show a greater development of the mesocone in the first lateral teeth (Lanzer & Veitenheimer-Mendes, 1985; Ohlweiler & Lanzer, 1993; Santos, 1994, 2003), whereas in *Anancylus rosanae* the most developed cusp is the endocone. The species described herein has endocones in the marginal teeth, while all cusps are well developed in *A. obliquus* and, conversely, they are very poorly developed in *U. concentricus*.

The dorsal and lateral plates of the jaw of *Anancylus rosanae* reveal no differences between them (only a small difference in size) as can also be observed in *Ferrissia rivularis* (Say, 1817) (Hubendick, 1964). However, the new species has low and wide plates compared to the large, tall plates of *F. rivularis*. In other species of Ancyliidae (*A. obliquus*, *A. dutrae*, and *L. vazi*), there is an obvious difference in size between dorsal and lateral plates. The free edge of the plates presents chitinous projections, a character not recorded in other species of Ancyliidae (Santos, 1989, 1994; Ohlweiler & Lanzer, 1993).

The reproductive system of *Anancylus rosanae* comprises a tubular ovotestis that differentiates it from other Ancyliidae that have a hemispheric ovotestis (Santos, 2003). The prostate of *Anancylus rosanae* is tubular and smooth, while in *Gundlachia*, *Uncancylus*, *Anisancylus* and *Hebetancylus* the prostate has protuberances and follicles (Santos, 2003). The flagellum is large and thick, as it is in *A. dutrae* (Santos, 1994), and it is better developed than in *Ferrissia* and *Gundlachia*. A long, tubular prolongation of the uterus can be seen in the female reproductive system, while in *A. obliquus*, *A. dutrae*, *U. concentricus* and *H. moricandi* the prolongation is short and in some cases it is wider (Santos, 1994, 2000; Ohweiler & Veitenheimer, 1995). Tubular prolongations of the uterus have not been mentioned in other species living in South America, such as *Lae-vapex vazi*, *Ferrissia gentilis* Lanzer, 1991, and *Burnupia ingae* Lanzer, 1991 (Santos, 1989; Lanzer, 1991). The poor development of the pseudobranch may be caused by the rapid and oxygenated water in which this species lives.

Anancylus differs from other members of the Ancyliidae mainly in possessing an anterior apex and tubular ovotestis; however, it shares with the members of this family the general arrangement of the adductor muscles and the reproductive system in general. For this reason, *Anancylus* is provisionally placed in this family until such a placement is confirmed or rejected by molecular studies.

ACKNOWLEDGEMENTS

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SYSTEMATIC REVISION OF *CHILINA* GRAY (GASTROPODA: PULMONATA) FROM NORTHWESTERN ARGENTINA AND DESCRIPTION OF A NEW SPECIES

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ABSTRACT

The family Chilinae in Argentina has been thought to include 17 species, most being found in Patagonia. All the original descriptions were based mainly on shell characteristics. The present work contains information about the shells, the radulae, and the reproductive and nervous systems in Chilinae species from northwestern region, along with the description of a new species. This new species from Tucumán Province is characterized by the shell and a penial morphology that includes pustules and transverse lamellae with irregular contours and longitudinal folds. The taxonomic position of *Chilina tucumanensis* is also reviewed and a redescription of *Chilina portillensis* is provided. Additional information is provided on their habitats and distributions. These records in northwestern Argentina now increase the total number of species of *Chilina* inhabiting Argentina to 19.

Key words: Chilinae, taxonomy, ***Chilina lilloi***, n. sp., freshwater snail, South America.

INTRODUCTION

The Chilinae (Gastropoda: Pulmonata) is one of the oldest freshwater families currently known. Such primitive features as the presence of one chiasmoneuric nervous system, horizontal lamellar tentacles, a noncontractile pneumostome, and an incomplete division of male and female ducts (Haeckel, 1911; Harry, 1964) have indicated a relationship to different groups of gastropods (Hubendick, 1945, 1978). Dayrat et al. (2001) published a molecular phylogeny of Euthyneura that argued for the monophyly of Hygrophila and proposed the Chilinae as a basal group. Klussmann et al. (2008) distinguished two clades within the Hygrophila, the first including *Chilina* Gray, 1828, and *Latia* Gray, 1850, and the second comprising higher limnic Basommatophora. More recently, Dayrat et al. (2011) on the basis of molecular analyses concluded that the Hygrophila was not a monophyletic group and proposed a relationship between the Chilinoidea and the Amphiboiloidea, although this contention was not well supported.

Chilinae is endemic in southern South America, extending from the coasts of Peru to Cape Horn (Pilsbry, 1911; Castellanos &

Gaillard, 1981) and also reaching the Falkland Islands (Brown & Pullan, 1987). This family consists in only a single genus, *Chilina*, with 32 nominal species, 17 of which are found in Argentina (Núñez et al., 2010). Most Argentine species of *Chilina* were originally described on the basis of shell characteristics alone, but more recently anatomical studies of certain species inhabiting Argentina were performed (Miquel, 1984, 1987; Ituarte, 1997; Gutiérrez Gregoric & Rumi, 2008; Gutiérrez Gregoric, 2010).

Chilina species can be found in quite different types of habitats – such as lakes, lagoons, dams, waterfalls, streams, rivers, canals, and estuaries; all generally with clean oxygenated water and variable temperature ranges (Gutiérrez Gregoric, 2008; Cuezco, 2009).

The northwest region of Argentina, extending between latitudes 21° and 30°S and longitudes 62° and 69°W, covers an area of approximately 470,184 km² and is an ecologic region characterized by the presence to four of the 14 ecoregions of Argentina (Puna, Altos Andes, Chaco, and Yungas). Northwestern Argentina and southern Bolivia constitute the boundary of the distribution of Andean Yungas forest within South America and forming a biogeographic

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as well as an ecologic and social unit (Grau & Brown, 2000). Of the freshwater gastropods, 13 species of pulmonates within the families Physidae, Planorbidae, Lymnaeidae, Ancyliidae, and Chilinae have been documented in northwestern Argentina (Rumi et al., 2008). Of the Chilinae, three species have been reported: *Chilina parchappii* (d'Orbigny 1835), *Chilina portillensis* Hidalgo, 1880, and *Chilina fluminea tucumanensis* Castellanos & Miquel, 1980. *Chilina parchappii* was recorded in Salta Province (Castellanos & Miquel, 1980) and is a species associated with lotic environments and mesohaline bodies of water of the southern pampas (Tietze & De Francesco, 2010). *Chilina portillensis*, cited in the Salta and Jujuy provinces, is considered endemic in Argentina, with a restricted and discontinuous distribution (Rumi et al., 2006; Núñez et al., 2010). *Chilina fluminea tucumanensis*, from Tucumán and Córdoba provinces, was established by Castellanos & Miquel (1980) as a subspecies of *Chilina fluminea* (Maton, 1809) based on specimens collected in Tucumán Province.

The aim of the present study was to provide information on the anatomy of the species of *Chilina* present in northwestern Argentina and to review the taxonomic status of all species of this genus in that area. Finally, during the examination of the collected specimens along with museum materials, a new species was identified. In this report, we therefore name this new member of the genus and present a complete description of the species.

MATERIALS AND METHODS

The specimens studied come from malacological collections at the Museo Argentino de Ciencias Naturales, Ciudad de Buenos Aires (MACN); the Museo de La Plata, Buenos Aires (MLP); and the Instituto Fundación Miguel Lillo, Tucumán (IFML). Additional material was collected during the field work carried out in northwestern Argentina during summer-autumn seasons from 2010 to 2011. Adult specimens collected were kept in 96% (v/v) aqueous ethanol, after a relaxation in menthol (12 h), followed by an immersion in hot water (70°C). For anatomical studies of the reproductive and pallial systems, the methodology proposed by Cuezco (1997) was followed. Dissections were made under a Leica MZ6 stereoscopic microscope and anatomical systems drawn with the help of a *camera lucida*. Terminology used for the anatomical descriptions follows

that of Harry (1964) and for the descriptions of the nervous system that of Ituarte (1997). Differences in lengths of the right and left nervous connectives were assessed by the Student t test (Gutiérrez Gregoric, 2010).

Radulae were separated from the buccal mass, cleaned by immersion in a sodium hypochlorite solution (Clorox™) and mounted for scanning electron microscopy. The radular-dentition formula gives the number of teeth per row: [(number of left and right teeth) / (number of cusps) + (number of central teeth) / (number of cusps)] number of transversal rows or their lower and maximum number. Seven shell measurements were taken according to Gutiérrez Gregoric (2010): total length (TL), length of the last whorl (LWL), aperture length (AL), total width (TW), aperture width (AW), aperture projection (AP), and spire length (SP). In order to obtain size-free variables to facilitate comparisons between different individuals and species, measurements of the soft parts were expressed as a proportion of the length of the last whorl.

SYSTEMATICS

Family Chilinae

Genus *Chilina* Gray, 1828

Diplicaria Rafinesque, 1833: 165.

Linneus d'Orbigny, 1835: 24, in part.

Dombeia d'Orbigny, 1843: 325: pl. 43, figs. 1–20.

Pseudochilina Dall, 1870: 357.

Acyrogonia Rochebrune & Mabilie, 1889: 25.

Type Species

Auricula (Chilina) fluctuosa Gray, 1828 (subsequent designation of Gray, 1847).

Original Diagnosis

"Shell ovate, thin; aperture large, expanded; columella flattened in front, with an oblique central fold; outer lip thin. Fresh running water".

Diagnosis

Oval (oblong to ventricose) shell with an expanded last whorl. Vestigial chiasmoneury. Incomplete division of male and female ducts. Calcareous granules in the vaginal lumen and presence of a secondary bursa copulatrix or accessory seminal receptacle. Penial terminal lamellas with cuticularized teeth-like structures.

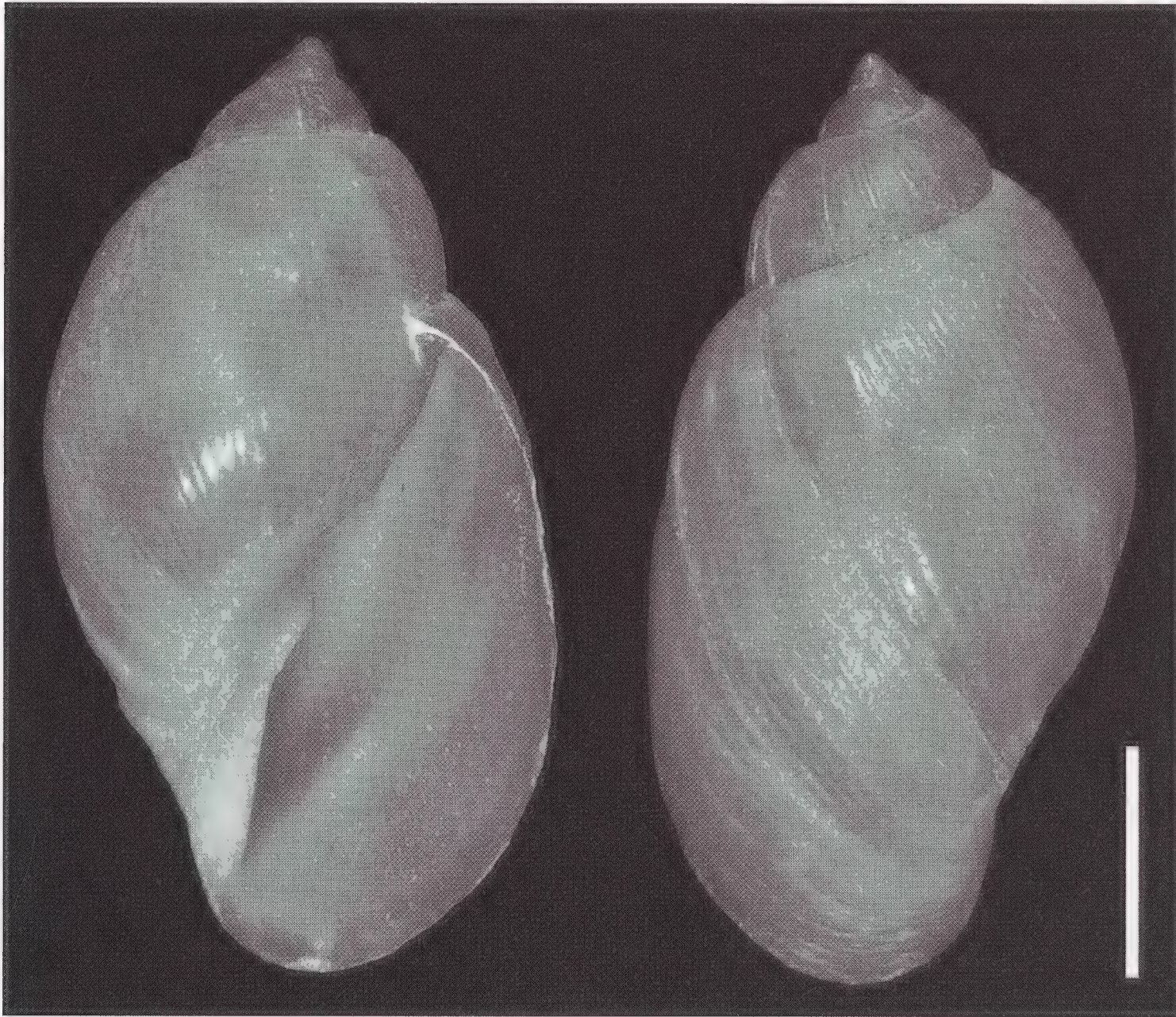


FIG. 1. Shell of *Chilina lilloi*, n. sp. (Holotype). Scale bar = 2 mm.

***Chilina lilloi*, n. sp.**

Type Locality

Juan Bautista Alberdi; unnamed stream on way to Escaba Dam; 27°40'06"S, 65°45'01"W; 1,580 m; Tucumán Province, Argentina.

Type Material

Holotype: IFML 15536 from type locality, 10/11/1999, M. G. Cuezco Leg (dry shell).

Paratypes: IFML 15537 from type locality, 10/11/1999, M. G. Cuezco leg (3 dry shells and 10 preserved specimens). MLP 13343 from type locality, 10/11/1999, M. G. Cuezco leg (3 preserved specimens).

Other Material Examined

Argentina, Tucumán Province: MLP 6887 (3 preserved specimens) and MLP 13343 (3 preserved specimens): Juan Bautista Alberdi Dept., unnamed stream on way to Escaba Dam; 27°40'06"S, 65°45'01"W; M. G. Cuezco leg.

Etymology

The name of the species was given after the scholar Miguel Lillo, from Tucumán.

Diagnosis

Shell with two columellar teeth, upper tooth situated in inner position and poorly developed. Penis sheath $1\frac{1}{4}$ times length of prepuce. Inner sculpture of penis sheath differentiated in three regions. Inner sculpture of prepuce with numerous longitudinal folds, smooth and tightly pressed. Gross nervous connectives. Right pleuroparietal connective preceding penis sheath.

Description

Shell (Fig. 1): Oval, light brown. Five convex whorls, each whorl larger than previous ones. Suture simple, deep not grooved. Protoconch and first whorl dark brown or, in some cases, almost reddish brown. Shell surface with pigmentated longitudinal bands, regularly arranged. Last whorl well developed, higher than

TABLE 1. Mean and range of seven measurements for *Chilina lilloi*, n. sp., *C. tucumanensis* and *C. portillensis* from Tucumán Province, Argentina. TL, total length; LWL, last whorl length; SL, spire length; AL, aperture length; TW, total width; AW, aperture width; AP, aperture projection; SL, spire length; n/d, no data.

Species		TL	LWL	AL	TW	AW	AP	SL
<i>Chilina lilloi</i> (n = 15)	Mean	10.93	9.82	8.07	6.22	3.99	1.31	0.63
	SD	0.84	0.79	0.57	0.39	0.20	0.36	0.05
	Max	12.37	11.22	9.11	6.70	4.34	2.39	0.74
	Min	9.62	8.42	6.80	5.16	3.64	1.06	0.58
<i>Chilina tucumanensis</i> (n = 42)	Mean	13.00	11.99	10.06	7.98	4.83	1.97	1.13
	SD	0.92	0.88	0.71	0.63	0.58	0.51	0.23
	Max	14.53	13.72	11.48	9.96	6.25	3.80	1.39
	Min	11.70	10.70	8.90	6.94	4.22	3.17	0.81
<i>Chilina portillensis</i> (n = 4)	Mean	n/d	10.97	9.52	7.48	5.35	2.31	n/d
	SD	n/d	1.67	1.26	1.03	0.37	0.21	n/d
	Max	n/d	13.24	11.21	8.84	5.89	2.60	n/d
	Min	n/d	9.23	8.19	6.36	5.10	2.16	n/d

wide. Aperture oval, slightly excavated at outer edge; aperture basal edge reddish, with smooth expansion outwards. Parietal callus whitish, thick, with its terminal portion slightly widened, flattened. Two columellar teeth, lower tooth more prominent and developed than upper, innermost on columella. Dimensions of 15 specimens taken from the material collected (Table 1).

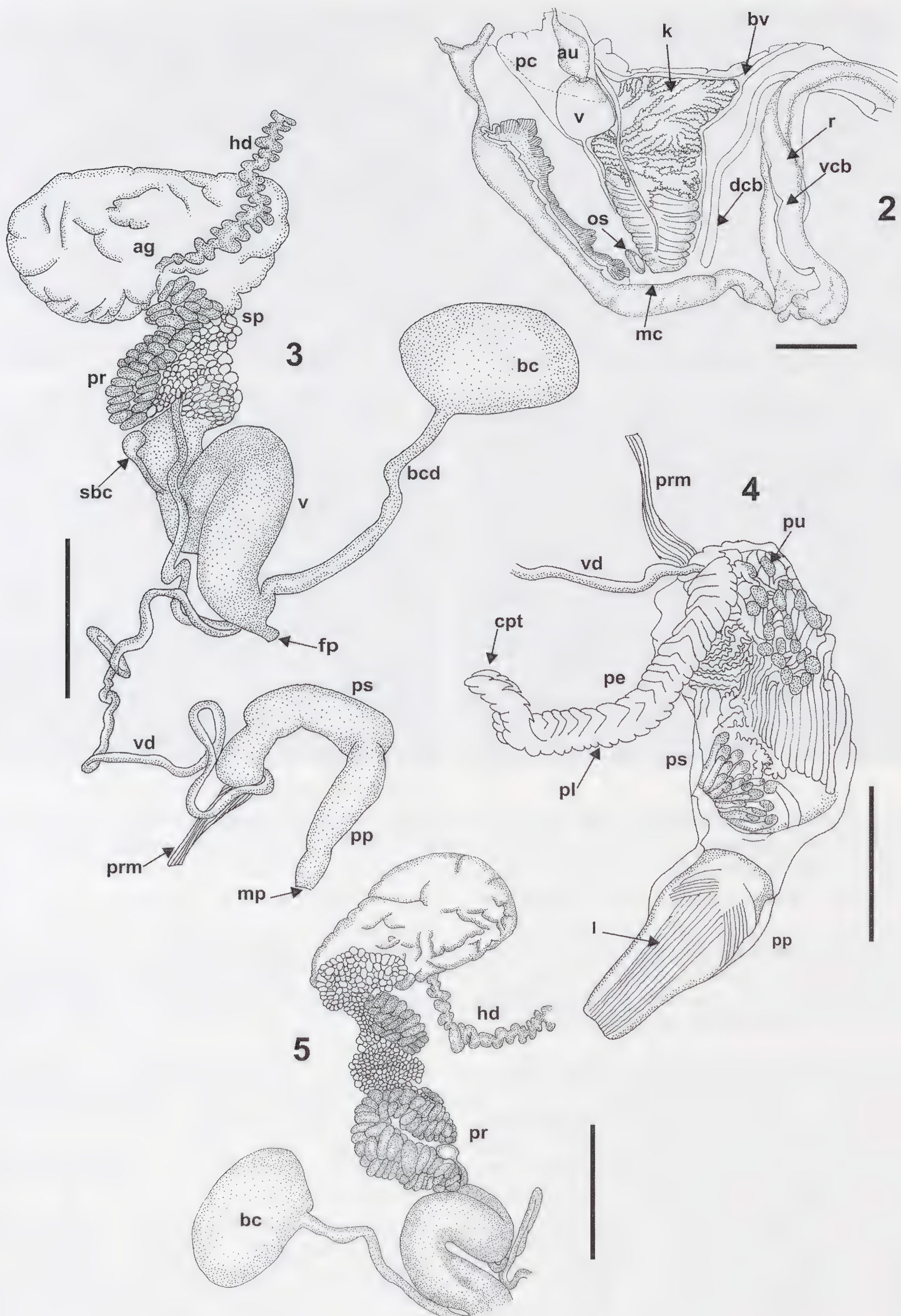
Pallial System (Fig. 2): Extending to half of body whorl. Pulmonary roof pigmented, uniform dark gray color. Kidney occupying almost entire pulmonary roof length. Kidney inner sculpture with numerous trabeculae, irregular contour, transverse, anastomosing. Trabeculae lower portion fused, giving a lobed appearance. Renal pore situated at distal end portion of kidney level with pneumostome. True ureter absent. Pericardium pear-shaped with length ½ of kidney length. Pulmonary vein parallel to left margin of kidney without branching. Two blood vessels running through top and right margins of kidney. Pallial cavity lamella divided into dorsal and rectal portion, dorsal portion located on lung roof on right side of kidney, rectal portion ¾ of rectum length, running on rectum surface. Rectum on right side of mantle cavity running until pneumostome.

Reproductive System (Figs. 3–5): Ovotestis constituted by several branches of small, rounded follicles flowing into a common duct. Ovotestis embedded in digestive gland at apex

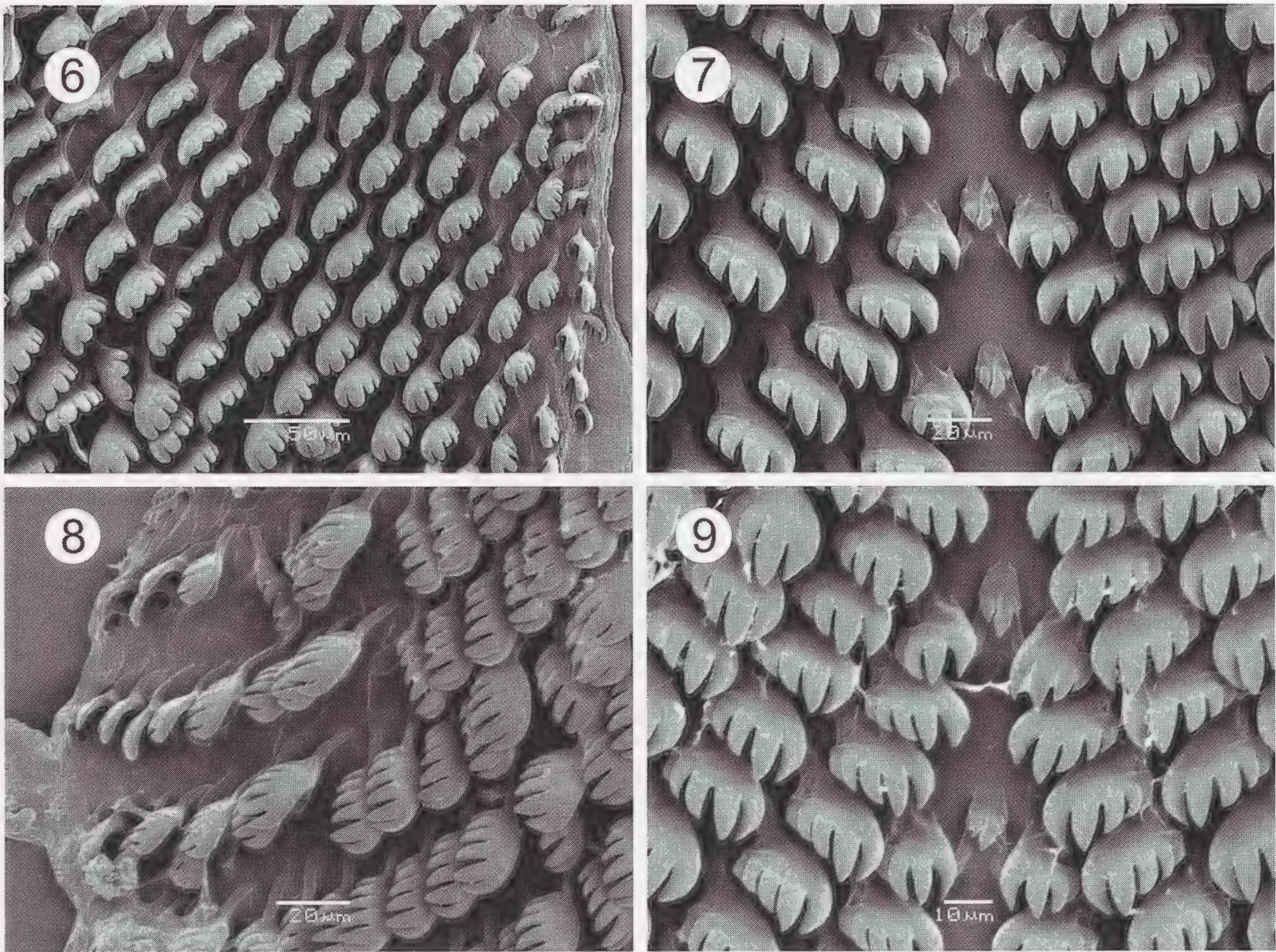
of shell. Common duct flowing into hermaphrodite duct, with irregular contours on both sides. Albumen gland elongated, glandular, twice as big as uterus and becoming sperm duct. Uterus proximal portion with glandular walls, folded over itself and on ventral part of albumen gland.

Female Genital System: Transparent, glandular-looking uterus wall continuing through free oviduct. Bursa copulatrix consisting of cylindrical tube with uniform diameter, inserting into distal free oviduct. Bursa copulatrix duct length 1½ times the diameter of its sac. Bursa copulatrix sac on left side of cephalopedal hemocoel between pericardial cavity and columella base. Secondary bursa copulatrix short, emerging from uterus base (25% bursa copulatrix duct length), cylindrical, expanded in its distal portion. Vagina shortest and narrow portion between free oviduct and female genital atrium. Vagina cylindrical, folded over free oviduct and entering female atrium. Female genital atrium opening outward through female pore on right side of head above male pore.

Male Genital System: Prostate extending to lower half of uterus and consisting of variable size and cylindrical-shaped acini. Vas deferens, emerging from terminal portion of prostate, showing a constant diameter throughout its length and running parallel to right distal portion of oviduct to penis complex, over uterus. Vas deferens entering body wall along an external



FIGS. 2-5. *Chilina lilloi*, n. sp. FIG. 2: Ventral view of pallial system dissected out. Scale bar = 2 mm; FIG. 3: Dorsal view of reproductive system. Scale bar = 1 mm; FIG. 4: Ventral view of reproductive system without ovotestis; FIG. 5: Penis inner wall. Scale bar = 1 mm. Abbreviations: ag, albumen gland; au, auricle; bc, bursa copulatrix; bcd, bursa copulatrix duct; bv, blood vein; cpt, cuticular penis tooth; dcb, dorsal ciliar band; fp, female pore; hd, hermaphrodite duct; k, kidney; l, lamellas; mc, mantle collar; mp, male pore; os, osphradium; pc, pericardic cavity; pe, penis; pl, penis lamella; pp, preputium; pr, prostate; prm, penis retractor muscle; ps, penis sheath; pu, pustules; r, rectum; rp, renal pore; sbc, secondary bursa copulatrix; sp, spermatheca; va, vagina; vcb, ventral ciliar band; vd, vas deferens; ve, ventricle.



FIGS. 6–9. Radula of *Chilina lilloi*, n. sp., from stream on way to Escaba Dam, Tucumán, Argentina. FIGS. 6, 8: Lateral teeth. Scale bars = 50 µm and 20 µm; FIGS. 7, 9: Central tooth and first lateral teeth. Scale bars = 20 µm and 10 µm.

TABLE 2. Ratio between ganglia length and last whorls length in *Chilina lilloi*, n. sp. (n = 7), *Chilina tucumanensis* (n = 4) and *C. portillensis* (n = 4). Abbreviations for each ganglion: c, cerebral; lc, left cerebral; lp, left parietal; lpe, left pedal; lpl, left pleural; p, pedal; rc, right cerebral; rp, right parietal; rpe, right pedal; rpl, right pleural; si, subintestinal; v, visceral.

	<i>Chilina lilloi</i>			<i>Chilina tucumanensis</i>			<i>Chilina portillensis</i>		
	Ratio	Mean (mm)	SD	Ratio	Mean (mm)	SD	Ratio	Mean (mm)	SD
lc – rc	13.11	1.28	0.14	16.70	1.86	0.44	13.46	1.48	0.21
lpe – rpe	3.68	0.36	0.04	4.99	0.56	0.08	3.85	0.42	0.03
lc – lpl	3.97	0.39	0.09	7.26	0.81	0.30	6.98	0.77	0.03
rc – rpl	3.26	0.32	0.13	6.20	0.69	0.25	5.34	0.59	0.04
c – p	8.37	0.82	0.23	10.70	1.19	0.36	6.74	0.74	0.17
rpl – rp	11.55	1.13	0.23	13.79	1.54	0.31	16.24	1.78	0.42
lpl – lp	2.47	0.24	0.13	4.52	0.50	0.09	4.99	0.55	0.20
lp – si	15.92	1.56	0.14	18.23	2.03	0.77	16.14	1.77	0.24
rp – v	16.19	1.58	0.40	20.90	2.33	0.15	19.85	2.18	0.25
si – v	4.86	0.48	0.30	3.63	0.40	0.05	6.55	0.72	0.11

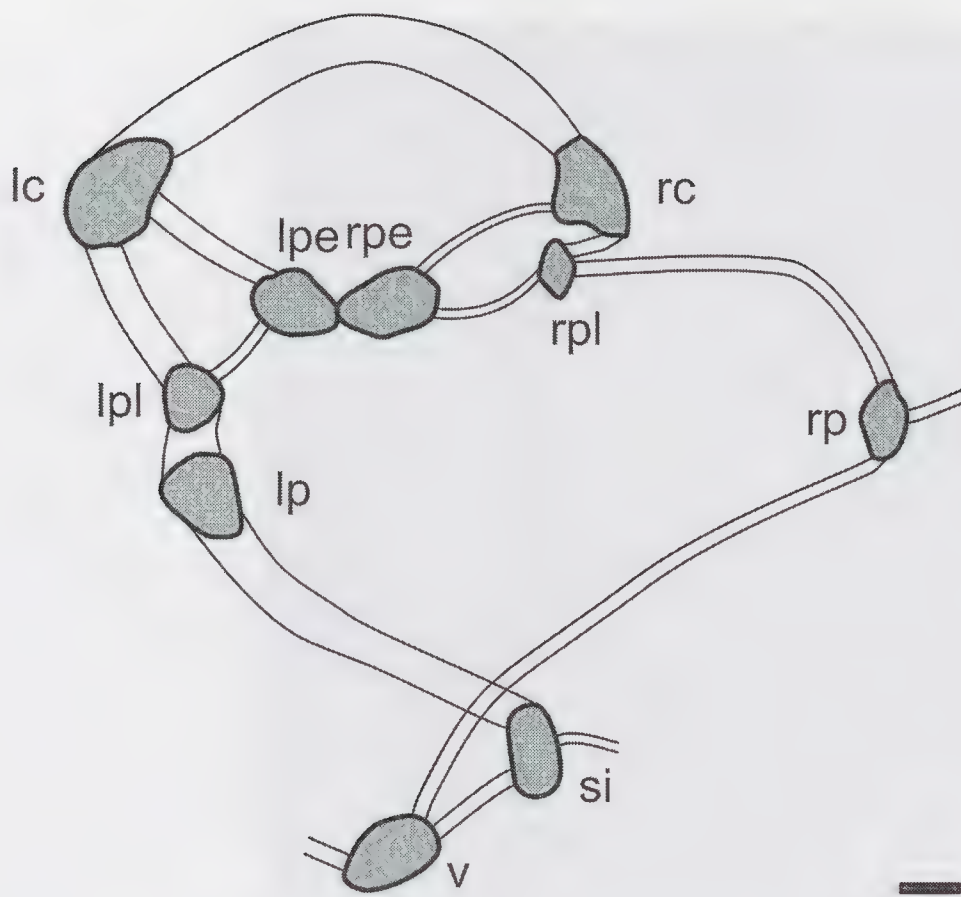


FIG. 10. Diagram of nervous system of *Chilina lilloi*, n. sp. Abbreviations: lc, left cerebral; lpe, left pedal; lp, left parietal; lpl, left pleural; rc, right cerebral; rpe, right pedal; rp, right parietal; rpl, right pleural; si, subintestinal; v, visceral. Scale bar = 1 mm.

partially occluded groove between female and male pores, subsequently reentering body cavity to be inserted into penis complex apex and folding into three loops of variable size before leaving body wall. Muscular penis sheath, $1\frac{1}{4}$ length of prepuce. Penis sheath inner sculpture differentiated into three regions. Upper right portion with pustules, upper left portion with irregularly contoured transverse lamellae, middle portion with longitudinal folds. Penis long ($1\frac{1}{4}$ length of penis sheath), robust, with outer surface cut by transverse lamellae, triangular in cross section. Prepuce cylindrical, thin, with constriction marked by oblique lines arranged in V making connection with penis sheath. Prepuce inner sculpture with numerous smooth, very tight longitudinal folds. Penis retractor muscle long, slender.

Digestive System

Radula (Figs. 6–9): Rows mean number 44 ($n = 4$; range = 42 to 46). Teeth per half row mean number (except for central tooth) 41 ($n = 4$; range = 39 to 43). Central tooth asymmetrical, tricuspid, elongated base as high as wide, mesocone more developed, slightly tilted to left. Cusps more developed giving tooth a pincer-like aspect. First lateral tooth tricuspid or tetracuspid, with endocone (tricuspid) or the inner second cusp (tetracuspid) more developed, base of tooth narrower than the apical part (cusp area), cusps taller than wide. On base of tooth, a groove dividing endocone from

other cusps. Second lateral tooth tetracuspid or pentacuspid, with inner cusp more developed, base of tooth narrower than apical part of tooth. Unlike previous tooth, base more notable. Last teeth with thin base, can even have 5–7 cusps of similar development.

Nervous System (Fig. 10; Table 2)

All connectives between ganglia relatively thick compared to both size of ganglia and system in general. Length of left connective joining cerebral ganglion with pleural ganglion greater than right connective, but the difference does not reach statistical significance (t_8 ; $p > 0.05$; 3.97 vs. 3.26% of LWL). Right pleuroparietal connective preceding penis sheath. Significant differences (t_8 ; $p < 0.001$) in length of pleuroparietal connectives, with left one smaller than right one (2.47 vs. 11.55% of LWL). Long connective (ratio: 15.92 of LWL) linking left parietal ganglion to subintestinal ganglion, located above posterior half of columellar muscle. Long connective (ratio: 16.19 of LWL) linking right parietal ganglion to visceral ganglion. One very short connective (ratio: 4.86 of LWL) linking subintestinal ganglion to visceral ganglion and closing posterior nerve ring.

Distribution & Habitat

Chilina lilloi was detected only at type locality in the Escaba area, Juan Bautista Alberdi Department in Tucumán; this locality is classified within the Yungas biogeographic ecoregion. The new species has been collected from a subtropical mountain stream with a stony substratum.

Remarks

Chilina lilloi is similar to the genus type species, *C. fluctuosa* (Gray, 1828), in general shell morphology (Haeckel, 1911). It differs from the latter in the presence of two small teeth on the columellar side in the aperture, those being absent in *C. fluctuosa*. In the present study, the dissection of several specimens showed three different types of sculpture. Pustules, lamellae, and longitudinal folds are present in the inner penis-sheath wall of *C. lilloi*. On the contrary, Harry (1964) described numerous papillae, some of them fused into lamellae present in *C. fluctuosa*. Other differences are the numerous, slender longitudinal folds found in the prepuce of *C. lilloi* that are not present in *C. fluctuosa* according to Harry (1964). *Chilina lilloi*, n. sp., differs from *C. tucumanensis* in having different shell morphology and a completely different sculpture pattern of the penis sheath.



FIG. 11. Shells of *Chilina tucumanensis*. Scale bar = 2 mm.

Chilina tucumanensis
Castellanos & Miquel, 1980

Chilina fluminea tucumanensis Castellanos & Miquel, 1980: 172.

Diagnosis

Male reproductive system with vas deferens forming two overlapping circles, of different sizes. Penis complex cylindrical, elongated. Penis sheath $2\frac{1}{4}$ times the length of prepuce.

Type Locality

Cochuna River, Tucumán Province, Argentina.

Type Material

Holotype: MACN 9927: from type locality, December 1916, Doello Jurado, M. leg (dry shell).

Paratypes: MACN 9927 (3 dry shells) and MLP 4042: from type locality, December 1916, Doello Jurado, M. leg. MACN 9954: Córdoba, Río Primero, 1917, Bondemberder leg (45 dry shells).

Other Material Examined

Argentina, Tucumán Province: IFML 15540: Concepción Dept; Las Cañas River; Villa

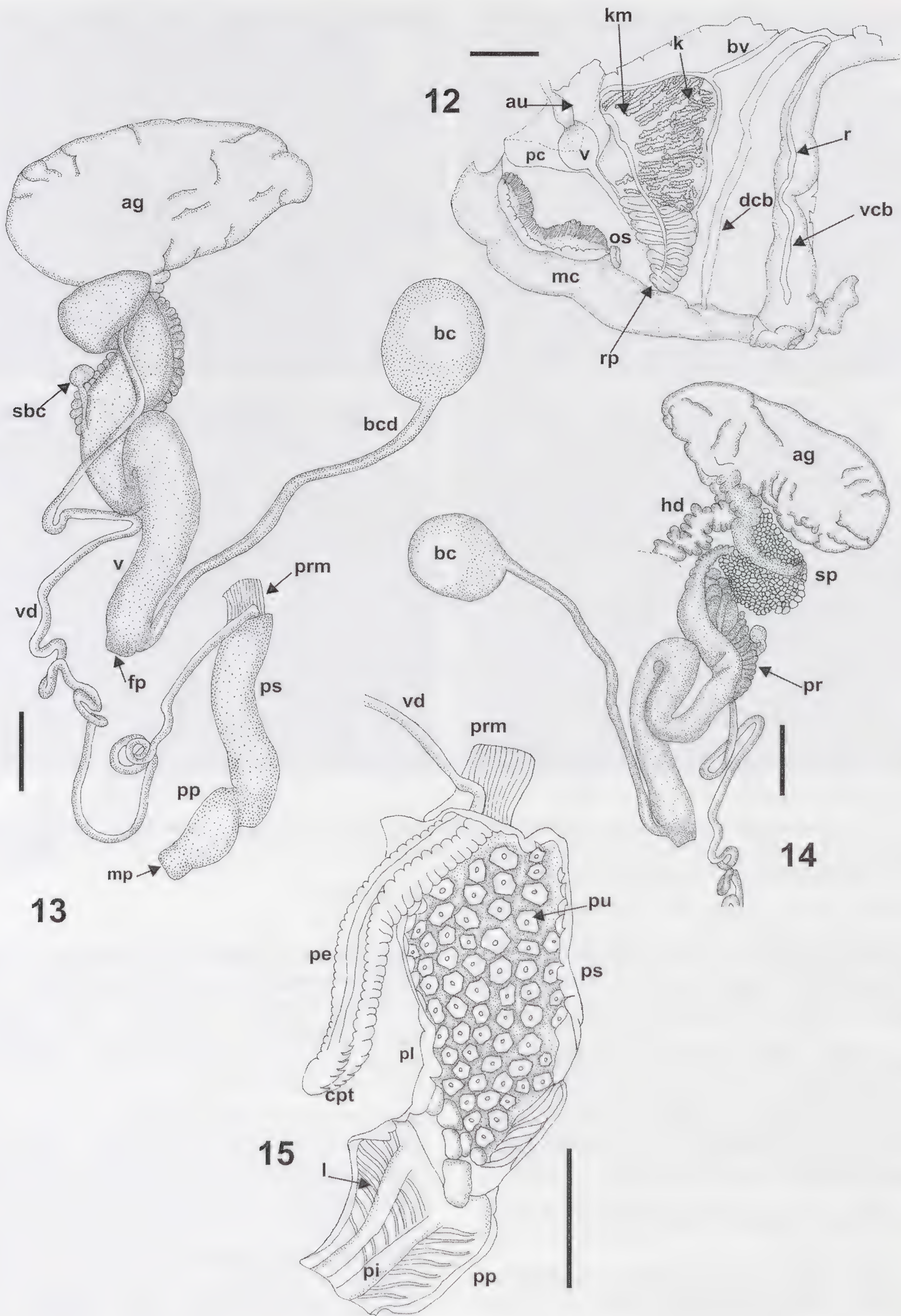
Lola Dam; 27°21'40"S, 65°49'13"W; 655 m; 14/11/2006; Cuezco M.G. leg. (23 preserved specimens). IFML 15541 (55 preserved specimens and 15 dry shells) and MLP 13344 (4 preserved specimens): Concepción; Cañas River; Villa Lola Dam; 09/07/2010, Ovando, X. M. C. leg. IFML 15550: Tafi del Valle, stream on the way to Tafi del Valle village; 27°01'45.3"S, 65°39'20.6"W; 1220 m; 28/03/2011; Ovando, X.M.C. leg. (60 preserved specimens). MACN 9927: Concepción, Cochuna River, 12/1916, Doello Jurado, M. leg (87 dry shells).

Etymology

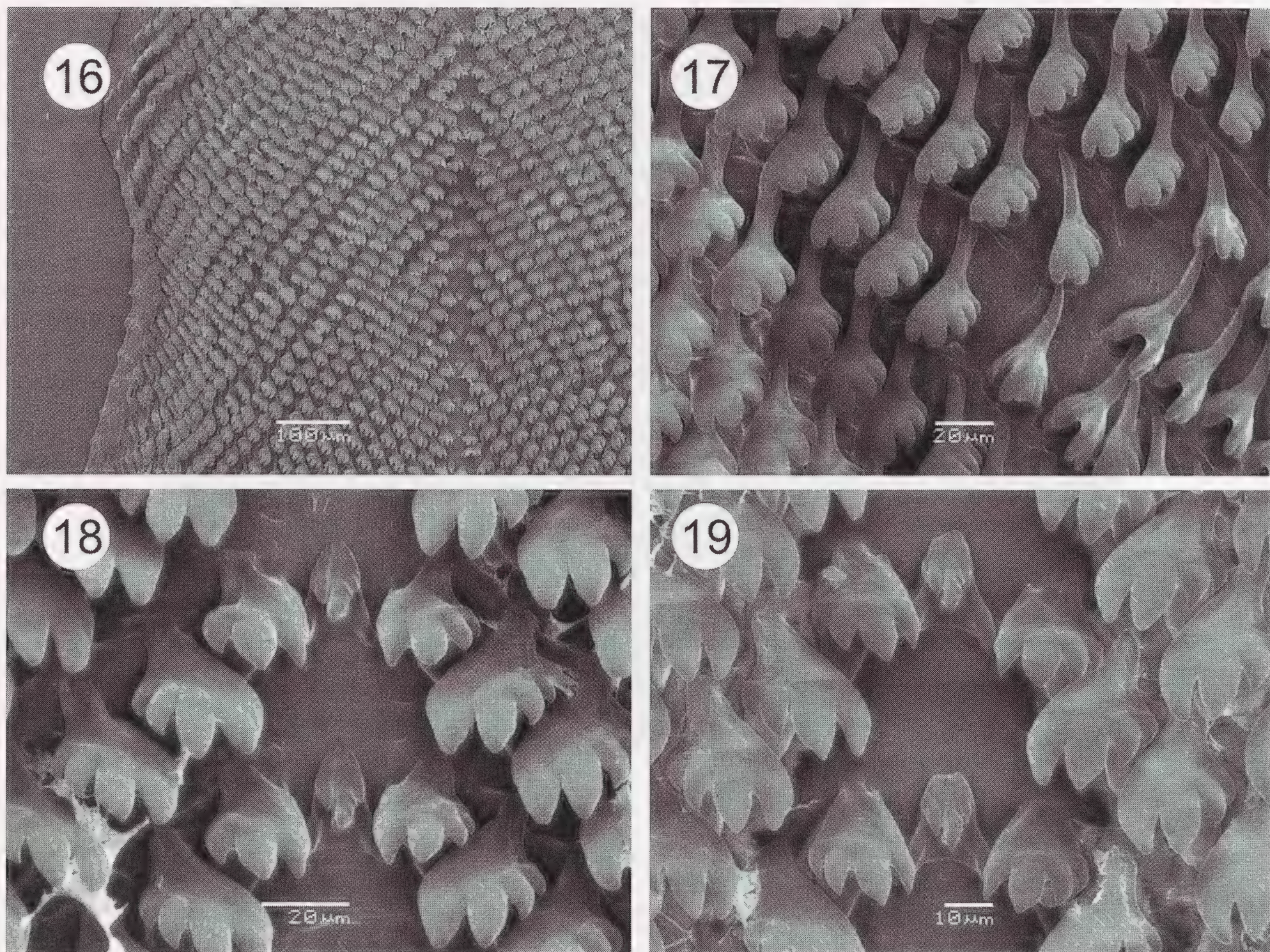
Named for Tucumán Province.

Description

Shell (Fig. 11): Dark brown, with dark brown bands in zigzags or spirals, more evident on the body whorl. Four convex whorls separated by simple suture. Protoconch sometimes eroded. Shells surface with growth lines, tight radial grooved layout. Body whorl prominent, not gibbous, longer than wide. Aperture oval, wider than long. Parietal area smooth, without ridges. Parietal callus wholly whitish, thick, narrow, with fold in the middle, posterior end slightly flattened. Two columellar teeth highly developed.



FIGS. 12–15. *Chilina tucumanensis*. FIG. 12: Ventral view of pallial system dissected out. Scale bar = 2 mm; FIG. 13: Dorsal view of reproductive system. Scale bar = 1 mm; FIG. 14: Ventral view of reproductive system without ovotestis; FIG. 15: Penis inner wall, Scale bar = 1 mm. Abbreviations: ag, albumen gland; au, auricle; bc, bursa copulatrix; bcd, bursa copulatrix duct; bv, blood vein; cpt, cuticular penis tooth; dcb, dorsal ciliar band; fp, female pore; hd, hermaphrodite duct; k, kidney; km, muscle band of kidney; l, lamellas; mc, mantle collar; mp, male pore; os, osphradium; pc, pericardic cavity; pe, penis; pi, pilasters; pl, penis lamella; pp, preputium; pr, prostate; prm, penis retractor muscle; ps, penis sheath; pu, pustules; r, rectum; rp, renal pore; sbc, secondary bursa copulatrix; sp, spermoviduct; va, vagina; vcb, ventral ciliar band; vd, vas deferens; ve, ventricle.



FIGS. 16–19. Radula of *Chilina tucumanensis* from Villa Lola Dam, Tucumán, Argentina. FIG. 16: General view. Scale bar = 100 µm; FIG. 17: Lateral teeth. Scale bar = 20 µm; FIGS. 18, 19: Central tooth and first lateral teeth. Scale bars = 20 µm and 10 µm.

The dimensions of 42 specimens from the collection of MLP were analyzed, including the type and paratype for MACN (Table 1).

Pallial System (Fig. 12): Pallial system dorsal portion light gray in whole area. Kidney triangular, occupying $\frac{3}{4}$ of pulmonary roof total length. Pericardium $\frac{1}{4}$ of kidney length, consisting of an auricle with a noticeable split.

Reproductive System (Figs. 13–15)

Female Genital System: Vagina cylindrical, longer than wide. Bursa copulatrix duct three times bursa sac diameter. Bursa copulatrix sac spherical-shaped. Secondary bursa copulatrix short, cylindrical, spherical (20% the length of bursa copulatrix duct), expanded in its distal portion.

Male Genital System: Prostate extending to lower half of uterus and composed of cylindri-

cal acini. Distal vas deferens folded forming two circles, overlapping; one being smaller near insertion of vas deferens into cylindrical, elongated penis complex. Penis sheath muscular, $2\frac{1}{4}$ length of prepuce, with slight convexity on right side. Penis sheath inner sculpture with regular pustules over entire surface. Pustules raised, polyhedral, with circular, white, well marked center. Pustules increased in size in area joining with foreskin and becoming ellipsoidal in shape. Penis elongated, almost as long as prepuce. Prepuce inner sculpture showing two longitudinal narrow pilasters, diagonal arranged in V at top and side of pilaster. Penis retractor muscle attached to short and stout columellar muscle.

Digestive System

Radula (Figs. 16–19): Rows mean number 53 ($n = 3$; range = 46 to 58). Teeth per half row mean number 39 (except for the central tooth) ($n = 4$; range = 36 to 43). Central tooth asym-

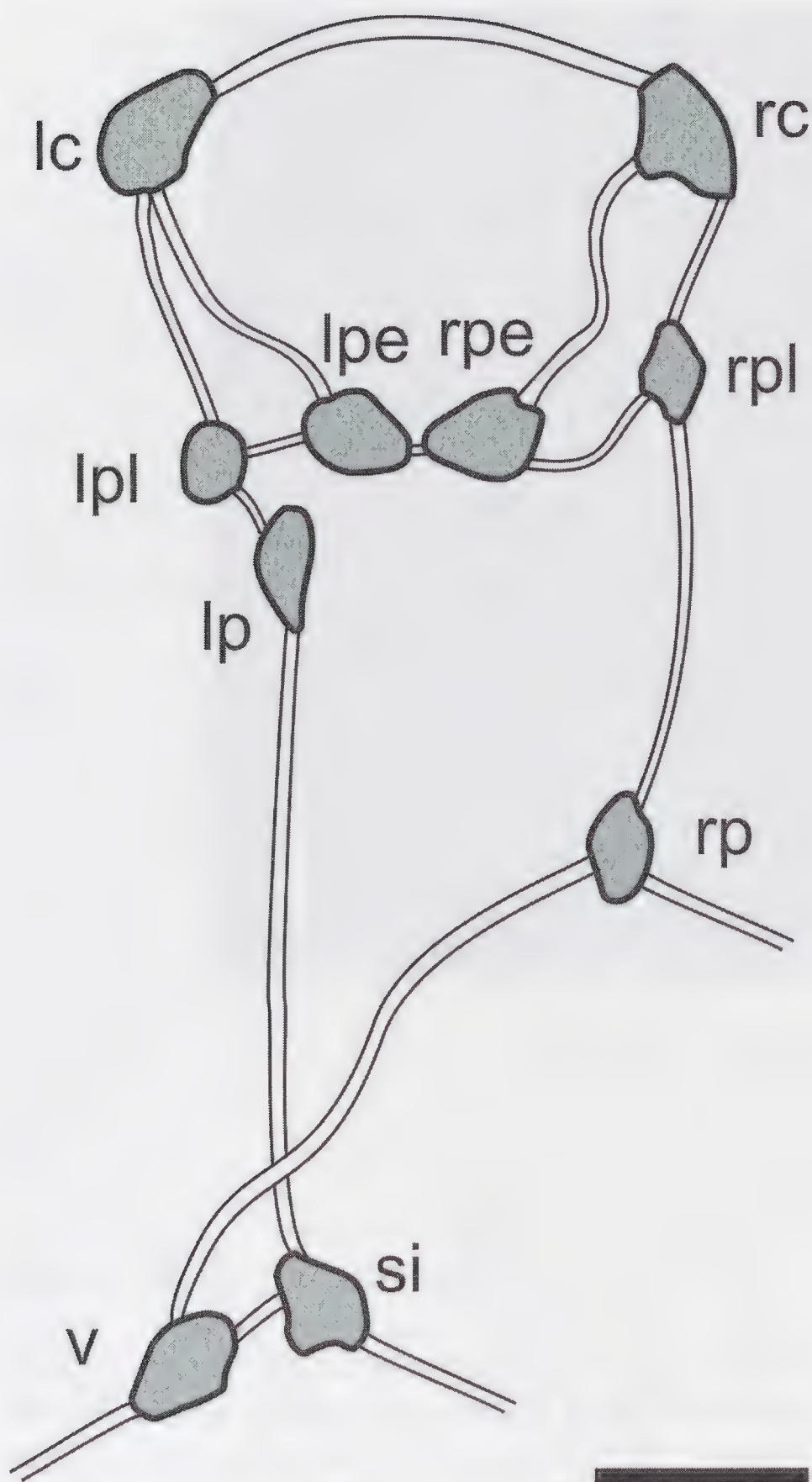


FIG. 20. Diagram of nervous system of *Chilina tucumanensis*. Abbreviations: lc, left cerebral; lpe, left pedal; lp, left parietal; lpl, left pleural; rc, right cerebral; rpe, right pedal; rp, right parietal; rpl, right pleural; si, subintestinal; v, visceral. Scale bar = 1 mm.

metrical, tricuspid, with triangular base as wide as high, mesocone most developed, sawlike edges in the two major cusps. First lateral tooth tetracuspid or tricuspid, with narrower base than apical part of tooth (cusp area), cusps wider than high, inner second cusp (tetracuspid) or mesocone (tricuspid) more developed. Second lateral tooth tetracuspid or tricuspid, with two inner cusps more developed, tooth base narrower than first lateral tooth. Last teeth having a thin base, can even have four to five cusps with similar development. Second inner cusp showing greatest development.

Nervous System (Fig. 20)

All connectives between ganglia relatively thin compared to both size of ganglia and system in general. Length of cerebral-pleural connective without significant statistical difference (7.3 vs. 6.2% of LWL; t_8 ; $p > 0.05$; Table 2). Right pleuroparietal connective running over the penis complex. Lengths of pleuroparietal connectives significantly different (4.52 vs. 13.79% of LWL; t_7 ; $p < 0.001$; Table 2). Parietal-subintestinal connective shorter than parietal-visceral connective (18.23 vs. 20.90 of LWL).

Distribution & Habitat

In addition to the known localities (Cochuna River in Tucumán and Río Primero in Córdoba), specimens were found on stones in two streams in the rain-forest area, the first with stony-sandy substratum and abundant macrophytes in the Las Cañas River and the other with sandy-stony substratum (Tucumán, Tafí del Valle).

Remarks

In the holotype and paratype material, two evident, well-developed columellar teeth were observed. In the rest of the specimens analyzed, however, the upper tooth does not show the same development and occupies an innermost portion of the columellar area. This last feature was also pointed out by Castellanos & Miquel (1980) in one of their figures. In the original description, the reproductive system was not mentioned. The analysis performed here showed differences between this species and *Chilina lilloi*, n. sp., with respect to both the general shape and the inner sculpture of the penis complex. The central tooth of the radula described by Castellanos & Miquel (1980) was tetracuspid with a larger development in one of lateral cusps; whereas in this specimen the tooth is tricuspid, with a more developed central cusp (mesocone).

Chilina portillensis Hidalgo, 1880

Chilina portillensis Hidalgo, 1880: 322; Smith, 1882: 846; Pilsbry, 1911: 547; Castellanos & Gaillard, 1981: 32 in part; Rumi et al., 2008: 82 in part.



FIG. 21. Shells of *Chilina portillensis*. Scale bar = 2 mm.

Type Locality

Portillo Pass (Andean pass between Argentina and Chile).

Type Material

Syntype (MNHN 23108): from type locality, Paz & Membiela leg.

Other Material Examined

Argentina, Tucumán Province: IFML15538 (22 dry shells and 25 preserved specimens) and MLP 13345 (4 preserved specimens): Concepción Dept., Potrerillo stream; 27°21'6.04"S, 65°50'8.24"W; 706 m; Ovando, X. M. C. leg.

Etymology

Named for Portillo, the Andean pass.

Diagnosis

Shell with two columellar teeth, a little apart. Penis sheath $2\frac{1}{4}$ times the length of the prepuce. Penis sheath inner sculpture displaying star-shaped pustules. Pustules arranged on left and right margins. Penis sheath middle region smooth, without sculpture. Prepuce inner sculpture with two short, broad pilasters.

Description

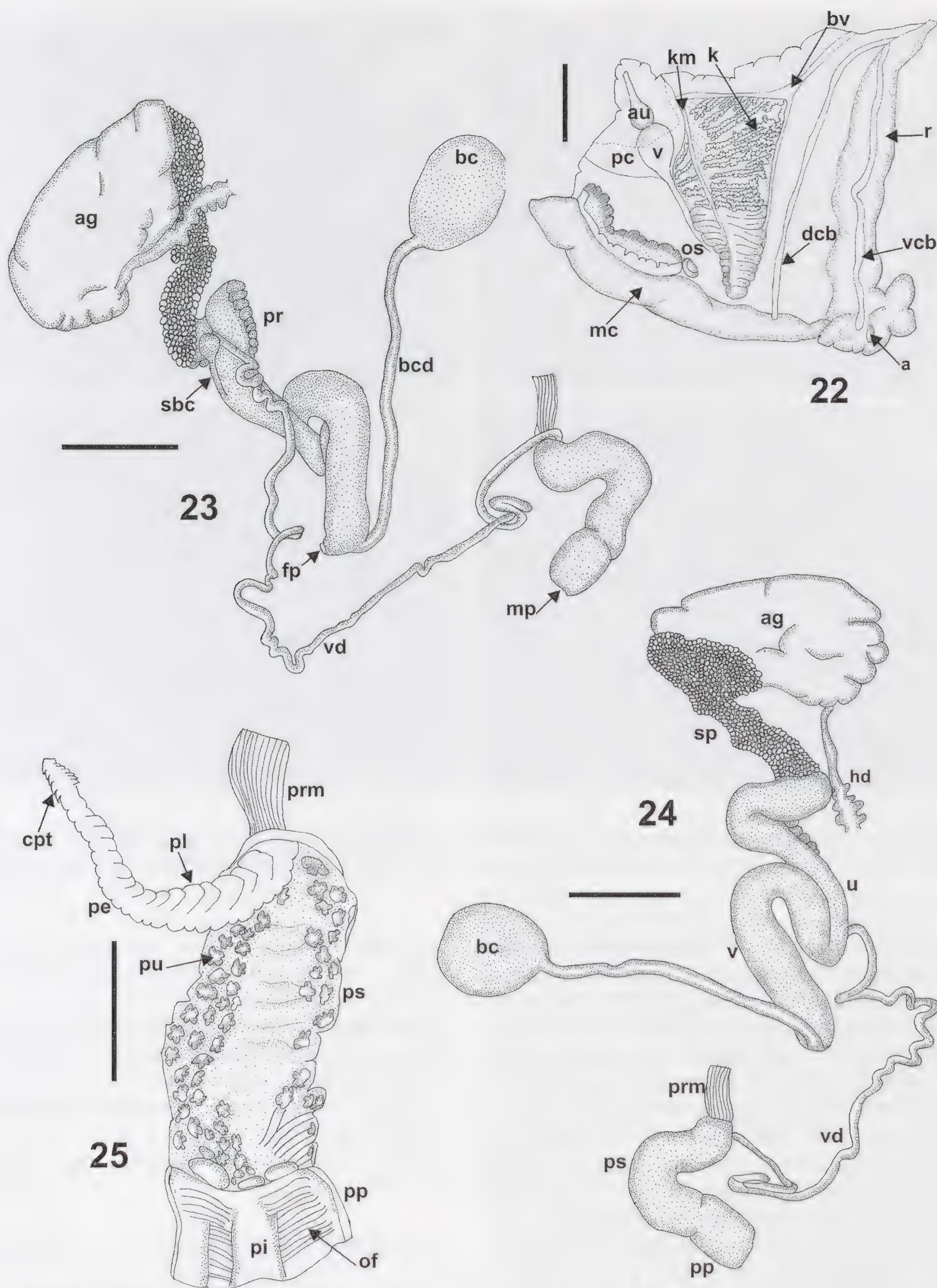
Shell (Fig. 21): Imperforate, ovate, rather solid, dull, almost opaque, smooth, olive-colored, with darker transverse bands slightly marked. Spire short, with eroded apex. Suture simple. Three, convex whorls, rapidly growing, last whorl somewhat distended. Aperture oval, pale reddish inside, with dark transverse lines. Columella thick, whitish, flattened at bottom. Two transverse columellar teeth slightly separated; lower tooth being larger than upper. Dimensions of four specimens of *C. portillensis* are shown in Table 1.

Pallial System (Fig. 22): Pallial system dark gray on entire surface. Mantle collar edge right margin with continuous line of light brown pigment. Kidney triangular, almost as long as whole lung-cavity roof. Pericardium $\frac{1}{2}$ kidney length.

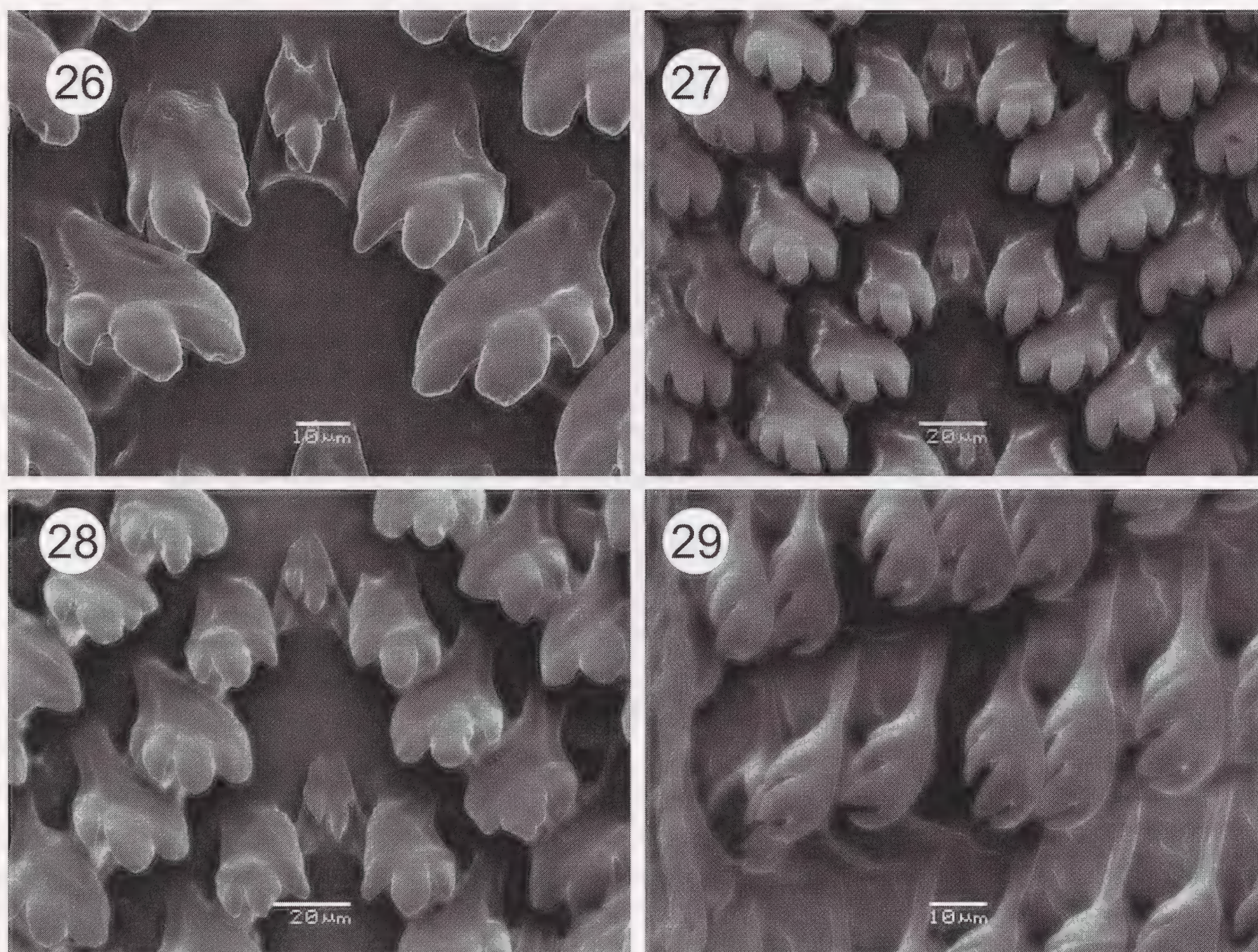
Reproductive System (Figs. 23–25)

Female Genital System: Bursa copulatrix duct length $3\frac{1}{4}$ times bursa copulatrix sac diameter. Secondary bursa copulatrix short, cylindrical, spherical (18% bursa copulatrix duct length), expanded in its terminal portion.

Male Genital System: Prostate extending to middle right side of uterus composed of cylindri-



FIGS. 22–25. *Chilina portillensis*. FIG. 22: Ventral view of pallial system dissected out. Scale bar = 2 mm; FIG. 23: Dorsal view of reproductive system. Scale bar = 1 mm; FIG. 24: Ventral view of reproductive system without ovotestis; FIG. 25: Penis inner wall. Scale bar = 1 mm. Abbreviations: a, anus; ag, albumen gland; au, auricle; bc, bursa copulatrix; bcd, bursa copulatrix duct; bv, blood vein; cpt, cuticular penis tooth; dcb, dorsal ciliar band; fp, female pore; hd, hermaphrodite duct; k, kidney; km, muscle band of kidney; mc, mantle collar; mp, male pore; os, osphradium; pc, pericardic cavity; pe, penis; pi, pilasters; pl, penis lamella; pp, preputium; pr, prostate; prm, penis retractor muscle; ps, penis sheath; pu, pustules; r, rectum; sbc, secondary bursa copulatrix; sp, spermoviduct; u, uterus; va, vagina; vcb, ventral ciliar band; vd, vas deferens; ve, ventricle.



FIGS. 26–29. Radula of *Chilina portillensis* from Potrerillo stream, Tucumán, Argentina. FIGS. 26–28: Central tooth and first lateral teeth. Scale bars = 10 µm and 20 µm; FIG. 29: Marginal teeth. Scale bar = 10 µm.

cal acini. Vas deferens sinuous all along, folding back on itself near insertion into penis complex. Penis complex cylindrical, curved down, inverted U-shaped. Penis sheath muscular, $2\frac{1}{4}$ prepuce length, with slight convexity toward right side. Penis sheath inner sculpture displaying star-shaped pustules arranged on penis sheath left and right margins. Penis sheath without sculpture on middle region. Pustules reduced in number to four or three, becoming ellipsoidal towards union with prepuce. Penis elongated, similar length to prepuce. Prepuce inner sculpture with two broad pilasters. Prepuce showing longitudinal grooves, oblique on both sides of pilasters.

Digestive System

Radula (Figs. 26–29): Rows mean number 54 ($n = 4$; range = 50 to 57). Teeth per half row mean 39 (except for the central tooth) ($n = 4$; range = 38 to 41). Central tooth asymmetrical,

bicuspid, triangular base, of equal length and width, central cusp more developed with serrate left edge. First lateral tooth tricuspid, with mesocone more developed, cusps equal in width and height. Second lateral tooth tricuspid or tetracuspid, with endocone and mesocone (tricuspid) or two inner cusps (tetracuspid) more developed. From tooth No 27 on, five cusps can be observed; the last two teeth, however, have four cusps.

Nervous System (Fig. 30)

All connectives between ganglia relatively thin compared to both size of ganglia and system in general. Length of cerebral-pleural connective significantly different (t_8 ; $p < 0.00004$) (6.98 vs. 5.34% of LWL; Table 2). Right pleuro-parietal connective running over penis complex. Lengths of pleuroparietal connectives significantly different (t_8 ; $p < 0.0002$), left one being smaller than right (4.99 vs. 16.24% of LWL).

DISCUSSION

This report provides anatomical and distributional information on the species of *Chilina* distributed in northwestern Argentina. On the basis of new anatomical information and the review performed, we have identified a new species within the genus, redescribed *Chilina portillensis*, and raised the subspecies *Chilina fluminea tucumanensis* to the rank of species.

Chilina parchappii, another species recorded from northwestern Argentina (Castellanos & Miquel 1980; Castellanos & Gaillard, 1981), has been found in only a single locality in Cachi, Salta Province (only two dry shells, one being damaged); whereas that species is very common and widely distributed in the southern pampas (Martín, 2003). Despite the exhaustive field work carried out in northwestern Argentina, we were unable to find any live specimens or dry shells and therefore could not further describe or provide new information on this species.

Castellanos & Miquel (1980) concluded that the differences in shell and radula morphology were not enough to consider *C. tucumanensis* at the species level. Nevertheless, in the present reevaluation we found new anatomical evidence to differentiate this taxon from *C. fluminea* (common species inhabiting the Del Plata Basin) at the species level. In *C. tucumanensis*, the vas deferens, before re-entering the haemocoelic space toward the penis complex, folds and forms two overlapping circles; whereas in *C. fluminea* the organ develops two or three large loops (Ituarte, 1997; Gutiérrez Gregoric, 2008). Another significant difference was found in the penis complex, being cylindrical and slender in *C. tucumanensis* but curved in *C. fluminea*. The penis sheath in *C. tucumanensis* is longer than the prepuce, whereas in *C. fluminea* the penis sheath can be almost the same length or else twice the prepuce (Gutiérrez Gregoric, 2008). Within the organization of the nervous system, the left subintestinal parietal connective in *C. fluminea* is longer than the right visceral one (Gutiérrez Gregoric, 2010), whereas in *C. tucumanensis* the reverse is true.

Furthermore, *C. lilloi*, n. sp., and *C. portillensis* can also be differentiated from *C. tucumanensis* by their radulae, nervous systems, and penis complex morphologies. The radula of

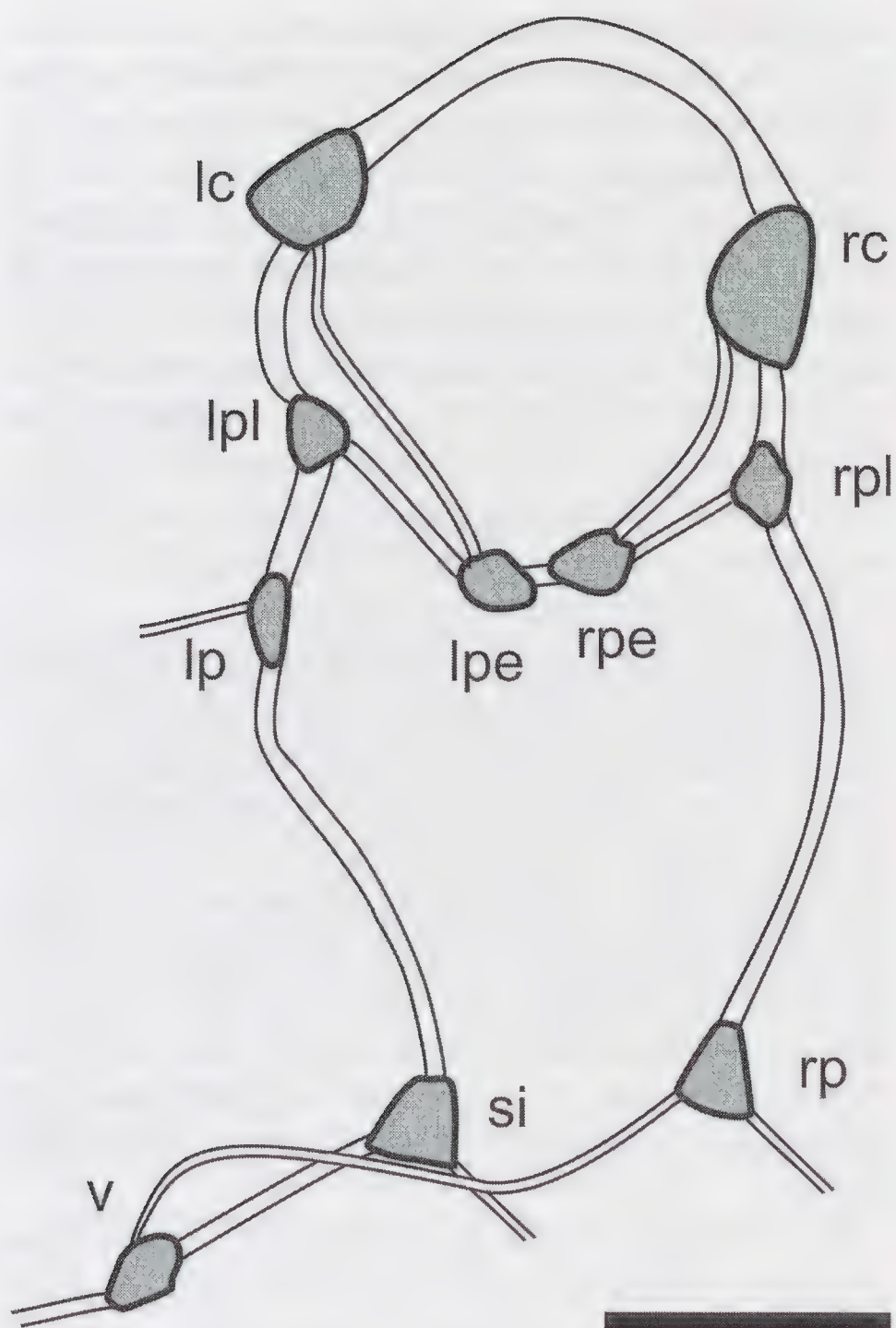


FIG. 30. Diagram of nervous system of *Chilina portillensis*. Abbreviations: lc, left cerebral; lpe, left pedal; lp, left parietal; lpl, left pleural; rc, right cerebral; rpe, right pedal; rp, right parietal; rpl, right pleural; si, subintestinal; v, visceral. Scale bar = 1 mm.

Distribution & Habitat

Chilina portillensis has been recorded in only one locality in the rain-forest area, Concepción Department in Tucumán. The specimen was collected from a stream with a stony substratum.

Remarks

Castellanos & Gaillard (1981), based on specimens deposited in La Plata Museum (MLP 2983 and 2967), mentioned the presence of *Chilina portillensis* in Jujuy and Salta provinces. Those materials have two well-developed columellar teeth, the spire well developed, and other proportions as in the description made by Hidalgo (1880).

C. lilloi, n. sp., has the lowest number of tooth rows (44) as yet described for any known species of the genus in Argentina (Gutiérrez Gregoric, 2010). The radulae of *C. portillensis* and *C. tucumanensis* are similar to each other with respect to both the number of rows and the shape of the teeth. In comparison to *C. fluminea*, however, the number of rows in the *C. tucumanensis* radula is greater, though in *C. fluminea* and *C. tucumanensis* the first lateral tooth has three or four cusps. The number of teeth per half row in *C. tucumanensis* and *C. rushii* Pilsbry, 1896, is the lowest recorded for species of *Chilina*. In addition, the last lateral teeth in *C. lilloi*, n. sp., *C. fluminea*, and *C. rushii* can have up to seven cusps (Gutiérrez Gregoric, 2010).

The penis complex in *C. lilloi*, n. sp., is curved and U-shaped, unlike that of *C. tucumanensis*, where the complex is cylindrical and elongated but never curved. Furthermore, in contrast to the conclusions of Miquel (1987) regarding the homogeneity of the penis-complex inner-wall sculpture, the present results show conspicuous differences among the species under consideration. In *C. lilloi*, n. sp., this sculpture is divided into three well-delimited portions, but in *C. tucumanensis* the inner wall shows only one type of sculpture, consisting in polyhedral pustules in an orderly array. By contrast, in *C. portillensis*, the inner-wall sculpture shows star-shaped pustule arrangements on the left and right margins around a smooth middle portion. Another anatomical difference found in the genitalia concerns the natural folding of the vas deferens, which in *C. lilloi*, n. sp. develops three small loops upon reentering the haemocoelic cavity toward the penial complex, but in *C. tucumanensis* develops two round, overlapping folds.

The organization of the pallial system was similar in all the species analyzed, and this finding is in agreement with previous descriptions provided by Harry (1969) and Brace (1983) for other *Chilina* species of South America.

All the species studied here have the same general pattern of the nervous system. In *C. megastoma* Hylton Scott, 1958, however, a slight swelling, not forming a true ganglion, between the left pleural ganglion and the sub-intestinal ganglion is present (Ituarte, 1997). Another difference observed between the species from the northwest and the Del Plata Basin is in the relative lengths between the left pleural and the parietal ganglia. *Chilina lilloi*, n. sp., shows the lower relative length (55% of the

length of *C. fluminea*). Furthermore, in *C. lilloi*, n. sp., as well as in *C. fluminea*, the length of the pleuro-subintestinal connective is larger than that of the pleuro-visceral one. In the rest of the species (*C. gallardoi* Castellanos & Miquel, 1980; *C. iguazuensis* Gutiérrez Gregoric & Rumi, 2008; *C. megastoma*; *C. portillensis*; *C. rushii*; and *C. tucumanensis*), the reverse is true (Gutiérrez Gregoric, 2010). In addition, in *C. lilloi*, n. sp., the right pleuro-parietal connective runs in front of the penis complex, whereas in the rest of the species that connective runs either over or behind the complex.

The findings from this study have increased the species richness of *Chilina* within the area from three to four and as a consequence have augmented the total number of species of this genus inhabiting Argentina to 19. In northwestern Argentina *Chilina* species were collected in rivers and streams of the rain forests called Yungas. This ecoregion is considered one of the most diverse in the country and has the highest values of plant and vertebrate taxa endemism from Argentina (Brown & Kappelle, 2001).

According to Gutiérrez Gregoric (2008), the family Chiliniidae shows a marked distribution pattern in Argentina, with the highest species richness and abundance in the southernmost areas (i.e., the Patagonia). In northwestern Argentina *Chilina* species richness is notably low, with only scarce localities of occurrences registered for the genus. With respect to the normal distribution gradient characterized by a decrease in freshwater-gastropod diversity and species richness with increased latitude (Rumi et al., 2006), the Chiliniidae appear to have an inverse pattern. Nevertheless, the findings documented in this study of new records for *C. tucumanensis*, *C. lilloi*, and *C. portillensis* enhance the information on the distribution of this family within areas constituting possible objectives for future biogeographical analyses.

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SPATIAL AND TEMPORAL VARIATION OF THE FRESHWATER MUSSEL *DREISSENA BLANCI* (WESTERLUND, 1890) LARVAE IN GREEK LAKES

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ABSTRACT

Recent molecular studies on dreissenid species in southern Europe found that *Dreissena blanci* (Westerlund, 1890) resides in several lakes where previous studies identified it as *Dreissena polymorpha* (Pallas, 1771). Because *D. blanci* seems to constitute a “new record” in the Balkans, the present study provides novel information on the biology and ecology of its planktonic larvae in four natural lakes and one reservoir located in western Greece. *Dreissena blanci* larvae were present in all lakes and their abundance varied between 3.4 and 440 ind. l⁻¹. The larvae were present all year round, having greater abundance in spring and summer and lesser in winter. The species seems to have an extended reproductive period from early spring until late summer. The larvae are distributed mainly in the upper 20 m of the deep lakes Amvrakia and Trichonis, while are aggregated close to or within the thermocline layer in the latter lake. A size-specific depth distribution was observed, with larger larvae residing in deeper strata during the stratification period. Temperature appears to be the most important parameter affecting the abundance variation of the larvae in the natural lakes, while water retention time is the major parameter in Stratos reservoir. In contrast, the soft, muddy substratum of Lake Lysimachia seems to be the inhibiting factor for the existence of a viable population here. The great similarities in larval ecology between *D. blanci* and *D. polymorpha* must be taken into consideration, as *D. blanci* could become another invasive species in freshwater ecosystems. Therefore, more research is required on the life cycle of this species, and the ecological and economic consequences of its presence in lakes and reservoirs of southeastern Europe.

Key words: *Dreissena blanci*, larvae, vertical, seasonal variation, Greek lakes.

INTRODUCTION

Recent molecular studies by Albrecht et al. (2007, 2009) and Wilke et al. (2010) on the biogeographical affinities and possible origins of the dreissenid mussels of the ancient Balkan lakes revealed two distinct sister species of *Dreissena* in the Balkans, *Dreissena presbensis* (Kobelt, 1915) and *Dreissena blanci* (Westerlund, 1890). Wilke et al. (2010) found *D. blanci* to be the only species occurring in the two large lakes of western Greece (Trichonis, Amvrakia) and also in the connecting reservoirs of Acheloos River, Kremasta and Stratos. *Dreissena blanci* was originally described from the area of the ancient Lake Trichonis, leading Wilke et al. (2010) to assume that this lake is probably its ancestral region. From this area, *D. blanci* presumably extended north, and today it occurs together with *D. presbensis* in Lakes

Prespa, Mikri Prespa and Pamvotis (Albrecht et al., 2007; Wilke et al., 2010).

Dreissena polymorpha was recorded in the Balkan lakes (Kinzelbach, 1992), while several other studies have also reported its presence in Greek rivers, natural lakes and reservoirs (Zarfdjian et al., 1990; Koussouris et al., 1994; Michaloudi et al., 1997; Kehayias et al., 2004; Kehayias et al., 2008; Doulka & Kehayias, 2008, 2011). Considering that *D. blanci* and *D. presbensis* are the only dreissenids present in several Balkan lakes, previous reports of *D. polymorpha* must be reconsidered. *Dreissena blanci* thus appears to constitute a “new record” for the Balkans, for which only limited information exists on its biology, ecology, and geographical distribution in the south Balkans.

Considering that *D. blanci* is closely related to the important invasive species, the zebra mussel *D. polymorpha*, and there are no previous

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reports on its larval stages, the present paper adds new data on the spatial and temporal variation of its planktonic larvae and their interrelation to environmental parameters in its presumed area of origin. The investigation of elements of this taxon's larval ecology compared to those of its relative *D. polymorpha* is expected to provide interesting aspects on the ecological significance of *D. blanci* in freshwater ecosystems, regarding also the possibility that it could become another invasive species.

METHODS

Study Area

The present study was conducted in four natural lakes and one reservoir of western Greece (Fig. 1). Among these aquatic ecosystems, Lake Trichonis is the largest natural lake in Greece, having a surface area of 98.6 km², a maximum depth of 57 m and a catchment area of 421 km² (Zacharias et al., 2002); previous

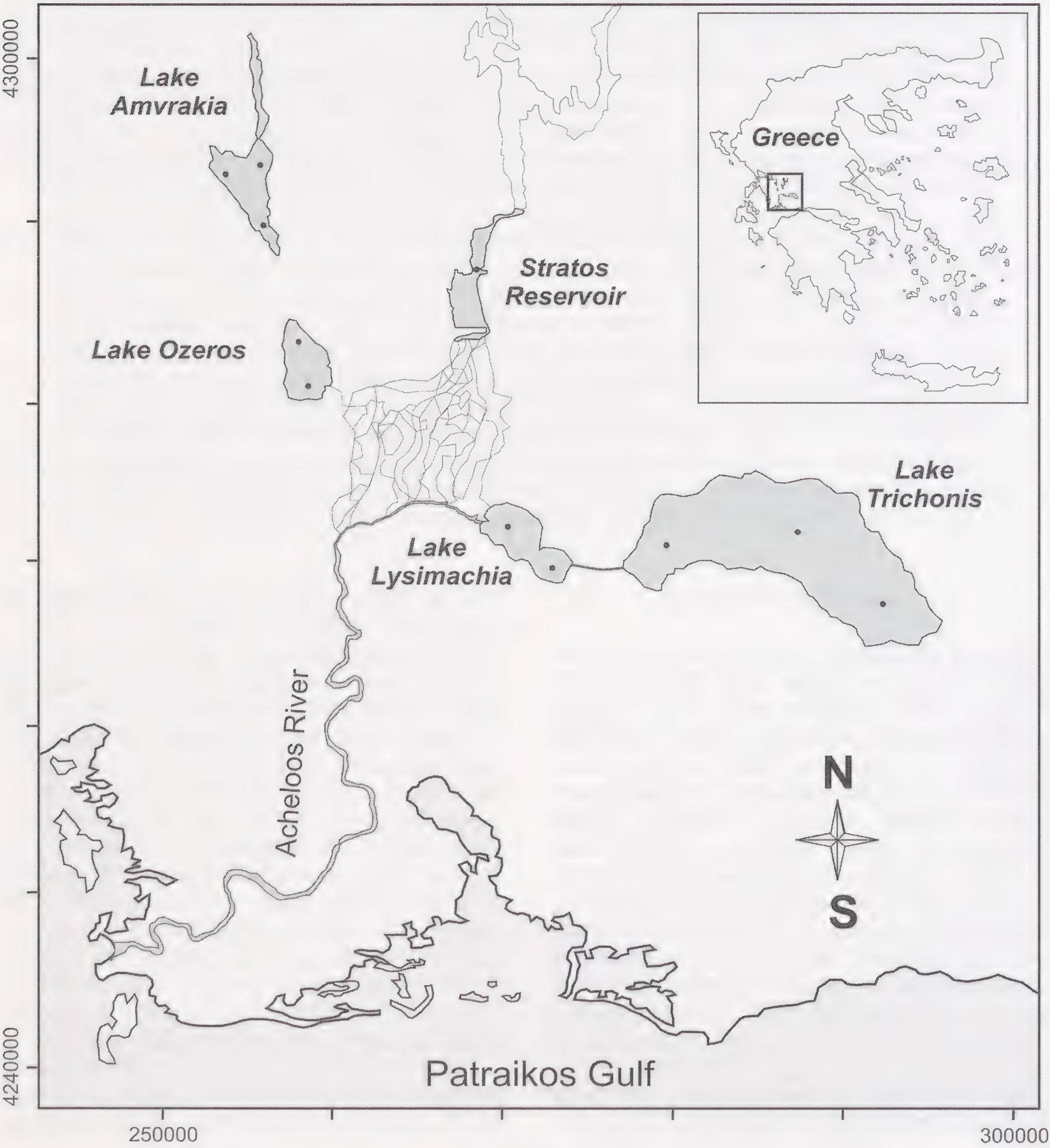


FIG. 1. Geographical location of the five lakes studied. Dots within each lake indicate the sampling sites.

TABLE 1. Sampling data for the zooplankton surveys conducted in the five water bodies.

Sampling area	Frequency/ Duration	Stations/ Depths	Net diameter/ porosity	Vertical hauls
Trichonis Lake	Monthly/24 months Sept. 2003–Aug. 2005	Three/ 48, 35, 25 m	40 cm/ 50 µm	at 10 m intervals
Amvrakia Lake	Monthly/24 months Sept. 2006–Aug. 2008	Three/ 45, 25, 23 m	20 cm/ 50 µm	at 5 m intervals
Lysimachia Lake	Monthly/12 months June 2009–May 2010	Two/ 8.1, 6.8 m	20 cm/ 50 µm	bottom to surface
Ozeros Lake	Monthly/12 months June 2009–May 2010	Two/ 5.6, 5.0 m	20 cm/ 50 µm	bottom to surface
Stratos Reservoir	Biweekly/24 months Sept. 2004–Aug. 2006	One/ 8 m	40 cm/ 50 µm	bottom to surface

studies have classified it as oligotrophic to mesotrophic (Skoulikidis et al., 1998; Doulka & Kehayias, 2008). Lake Amvrakia is a deep mesotrophic lake (maximum depth: 50 m, catchment area: 112 km²), which belongs to the sulphate type (Overbeck et al., 1982). The lake has strong water level fluctuations due to high evaporation rates, especially during the summer, and irrigation of the surrounding agricultural area. These variations usually result in the drainage of the shallower northern part of the basin (Fig. 1) in certain periods/years and, consequently, in the fluctuation of the total surface area of the lake, which ranges between 14 and 22 km². Both Trichonis and Amvrakia are warm monomictic lakes, exhibiting a long period of thermal stratification from May to October.

Lakes Lysimachia and Ozeros are smaller and shallower (maximum depth 8.1 and 5.6 m, respectively), with a surface area of 13.5 and 10.1 km². Lake Lysimachia has positive water balance due to inflow of water from the neighbouring Trichonis Lake, to which it is connected by a 6.5 km channel. There is also an outflow of water from the lake to the Acheloos River via a channel in its western area. Until 2000, the lake received the untreated urban wastewaters of the nearby city of Agrinio, with a population of about 80,000, and thus became eutrophic. Lake Ozeros is supplied with water from torrents that occur mainly to its east-southeast, and also through a channel from the Acheloos River, when it floods. Due to their ecological importance, all the above lakes are considered as special protected areas and have been listed as NATURA 2000 Greek sites. Their major environmental disturbance is the receiving of

agricultural drainage and wastes from nearby farms and villages. Finally, Stratos reservoir is the last of four reservoirs along Acheloos River and is situated between two dams, Kastraki (upstream) and Stratos (downstream). It has a surface area of 11 km², a maximum depth of 15.5 m and the water volume fluctuates between 60 and 70.2 X 10⁶ m³, resulting in a mean depth of between 5.5 and 6.4 m.

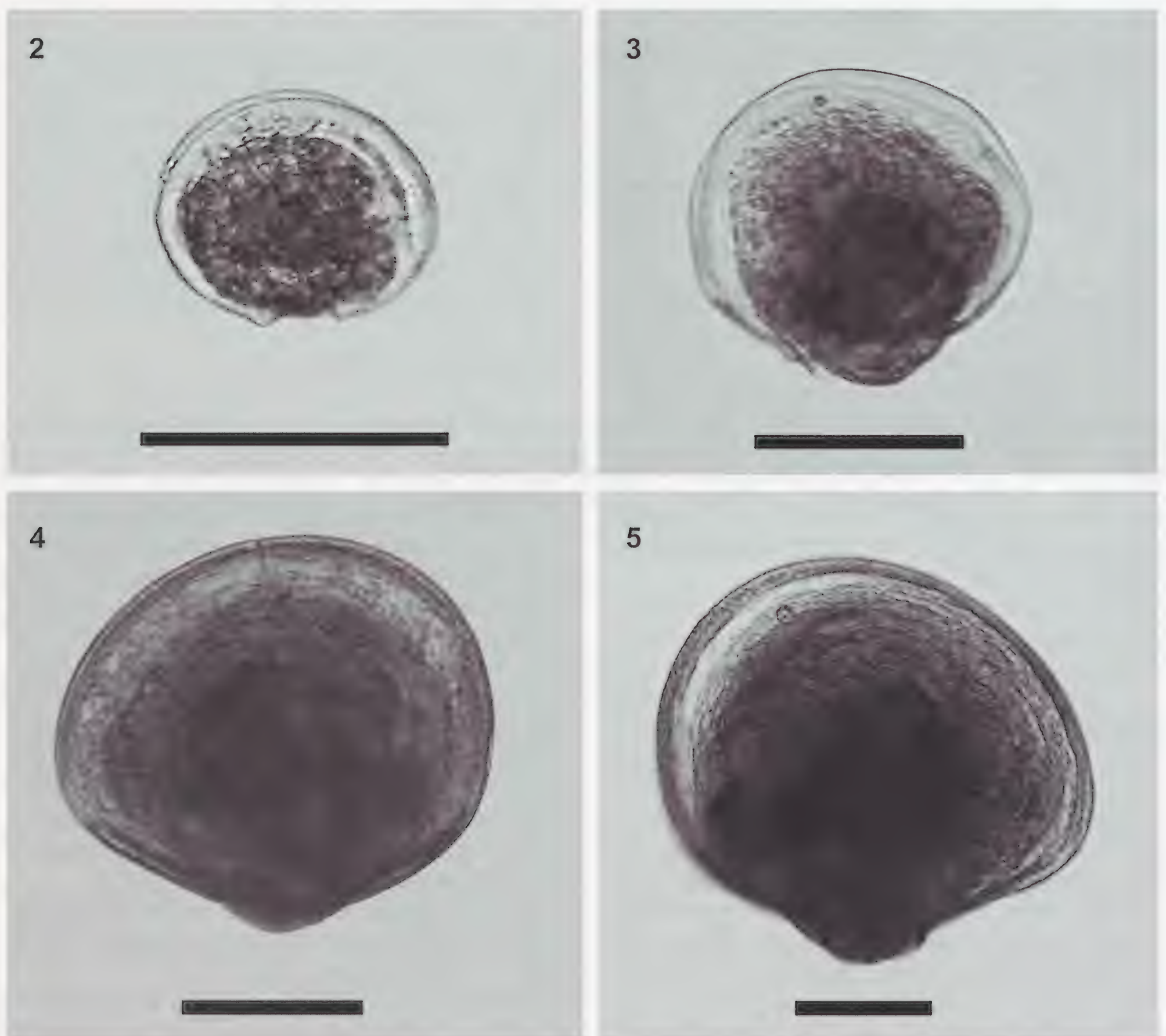
Zooplankton Surveys

Zooplankton sampling in the five lakes was conducted in various surveys of 12 and 24 months duration in different years (Table 1). The sampling was carried out using plankton nets of different dimensions but with the same porosity (50 µm), with vertical hauls from bottom to surface in the shallower lakes. Closing nets were used in the deep lakes Trichonis and Amvrakia to conduct sampling at depth intervals of 5 to 10 m (Table 1). In Stratos reservoir, the sampling was conducted from a bridge crossing the reservoir (Fig. 1). In all cases, the nets were towed at a speed of approximately 0.5 m sec⁻¹ and after collection all samples were preserved in 4% neutralized formalin solution, in a final volume of 100 ml.

During each zooplankton survey vertical profiles of temperature, oxygen concentration, pH and conductivity were taken from the surface down to 40 m, using portable WTW instruments in Lake Trichonis and Stratos reservoir, and a Troll 9500 (In-Situ Inc.) water quality multi-parameter instrument in lakes Amvrakia, Lysimachia and Ozeros. Water transparency was measured in all cases with a Secchi disc. To estimate total phosphorus (TP), phosphates

(PO_4), nitrates (NO_3), nitrites (NO_2), ammonia (NH_4) and silicates (SiO_2), water samples were collected at the deepest station from various depth intervals with a 5 L water sampler (Hydro-Bios free flow water sampler). Analyses of all chemical parameters were performed in the laboratory according to A.P.H.A et al., (1998). To determine chlorophyll-a concentration (chl-a), 1500 ml of the water samples taken from the above depths were filtered through a Whatman GF/A CatNo 1820-047 ($1.6\ \mu\text{m}$) glass fiber filter shortly after collection. Pigment extraction was made in 90% acetone and concentrations were determined spectrophotometrically using a BOECO S-20 spectrophotometer (A.P.H.A. et al., 1998).

For the abundance analysis of zooplankton, three counts of 1.5 ml subsamples from each sample were made on a Sedwick-Rafter cell having a total volume of 100 ml (Doulka & Kehayias, 2008). Length measurements were taken from all specimens of *Dreissena blanci* larvae in each sample using an optical micrometer fitted in a microscope. Four size classes (S1, S2, S3 and S4) were established for specimens having a body length less than $100\ \mu\text{m}$ (S1), for specimens of $100\text{--}200\ \mu\text{m}$ (S2), of $200\text{--}300\ \mu\text{m}$ (S3) and for specimens longer than $300\ \mu\text{m}$ (S4). Photographs of specimens of each of the four size classes are presented in Figures 2–5. Larvae smaller than $100\ \mu\text{m}$ are probably at the stage of entering the plank-



FIGS. 2–5. Photographs of *D. blanci* specimens. Scale bars = $100\ \mu\text{m}$. FIG. 2: Size class S1 ($< 100\ \mu\text{m}$); FIG. 3: Size class S2 ($100\text{--}200\ \mu\text{m}$); FIG. 4: Size class S3 ($200\text{--}300\ \mu\text{m}$); FIG. 5: Size class S4 ($> 300\ \mu\text{m}$).

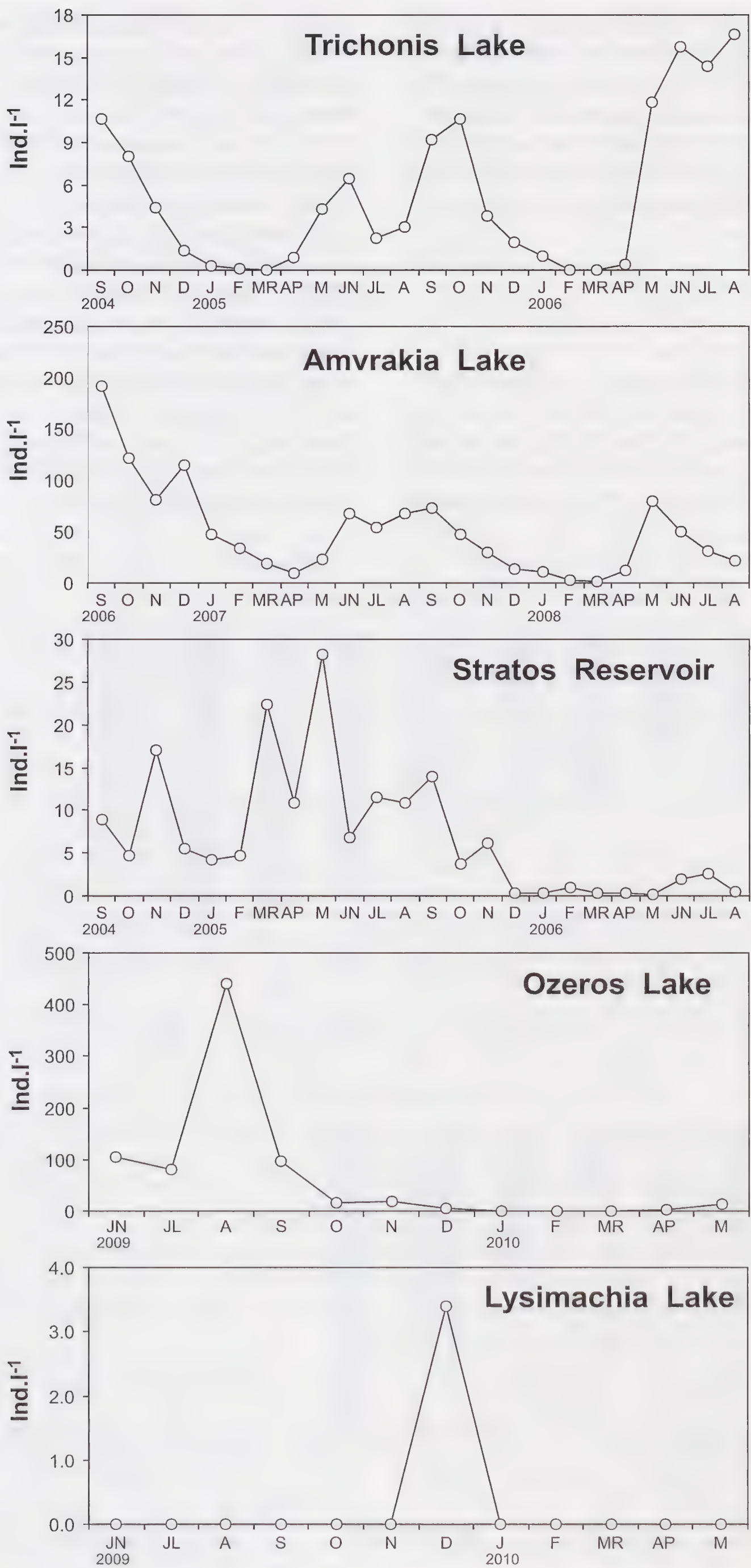


FIG. 6. Seasonal variation of the abundance (ind. l⁻¹) of *D. blanci* larvae in the five lakes studied. (In lakes Trichonis and Amvrakia the values correspond to the average abundance in the 0–20 m depth layer).

totrophic period, as reported by Sprung (1993) for *Dreissena polymorpha*. Similarly, larvae of S2 are considered in a pre-settling stage, while S3 and S4 larvae are at the settling stage.

To obtain comparable data of the vertical distributions of the *D. blanci* larvae, the weighted mean depth (WMD) was calculated for each species as follows:

$$WMD = \frac{\sum (NT_i \times T_i)}{\sum NT_i}$$

where WMD = weighted mean depth, NT_i = abundance in depth i , and T_i = depth (m). Although the weighted mean depth cannot represent the actual vertical distribution of a species, it is a good numerical base for the application of statistics. Thus, differences

between the WMDs of *D. blanci* larvae were tested using the non-parametric Kruskal-Wallis and Mann-Whitney (U) tests. The same tests were also applied to compare abundance and size distribution results. Multiple regression analysis was used to clarify the influence of the environmental factors on *D. blanci* larvae for each of the zooplankton surveys. The analysis was performed using forward stepwise selection to identify the significant factors. These factors were then correlated with each other (Pearson's r) to eliminate those having strong correlation. Finally, the explanatory factors were standardized so as to compare their relative importance. All data analyses were performed using the SPSS 17.0 (SPSS, Inc. 2008) statistical package.

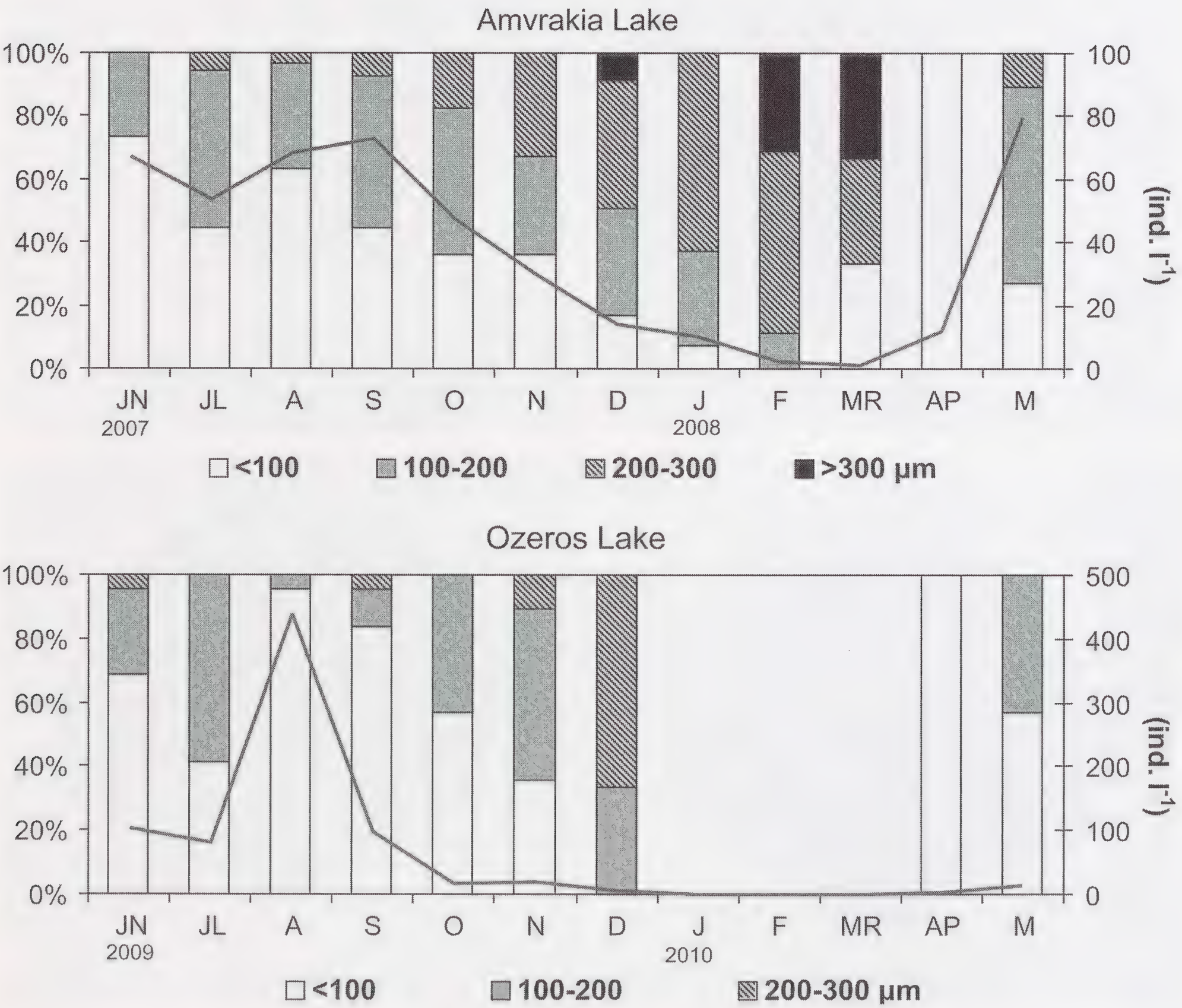


FIG. 7. Size distribution of *D. blanci* larvae in lakes Amvrakia and Ozeros expressed as percentages (%) of the four size classes S1 (< 100 µm), S2 (100–200 µm), S3 (200–300 µm) and S4 (> 300 µm) in the total population. Solid lines indicate the seasonal variation of the total abundance (ind. l⁻¹) of the larvae in each lake.

RESULTS

Geographic Distribution and Seasonal Variation

Dreissena blanci larvae were present in all the sampling sites, although there were considerable differences in their abundance between the five lakes. The highest abundance was recorded in Lake Ozeros (439.9 ind. l⁻¹ in August 2009) followed by Lake Amvrakia (194.2 ind. l⁻¹ in the 0–20 m layer in September 2006). In contrast, the lowest value was recorded in Lake Lysimachia, where a few specimens were found in only one occasion (December 2009). Concerning seasonal variation, *D. blanci* larvae had an almost continuous presence in the deep lakes Amvrakia and Trichonis and Stratos reservoir, while they were absent from Ozeros Lake samples from winter to early spring (Fig. 6). Generally, higher abundances were recorded during the warmer months of the year. The abundance variation pattern was similar for the deep lakes Amvrakia and Trichonis, where a decrease of abundance was recorded during autumn and winter, while greater abundances were found in spring and summer. Similarly, the highest abundance in Lake Ozeros was recorded in late summer, while in Stratos reservoir the highest abundance was recorded in May 2005 and the lowest in March 2006 (18.0 and 0.28 ind l⁻¹, respectively).

Differences in the abundance of the larvae between the two sampling years were recorded in Amvrakia Lake and Stratos reservoir. In Stratos, the larvae were significantly more abundant during the first sampling period (September 2004 to August 2005), than the second (September 2005 to August 2006) when their abundance was nearly four times lower (U-test, $p = 0.0001$). Similarly, in Amvrakia Lake, the larvae were more abundant from September 2006 to August 2007 than September 2007 to August 2008 (U-test, $p = 0.004$). In contrast, no such difference was detected in Lake Trichonis (U-test, $p = 0.773$).

Length measurements of the larvae found in Lake Amvrakia showed that the smallest S1 larvae were present almost all year round, and only in February 2008 were they absent from the samples (Fig. 7). S2 larvae were also present in most months except March and April 2008, while S3 were not present in the population in spring and early summer (Fig. 7). Finally, the larger S4 class larvae were found only from winter 2007 to early spring of 2008. The percentage contribution of S1 to the total larvae abundance was greater in spring while

the larger specimens of S3 and S4 had greater contribution in winter. The findings were similar in Lake Ozeros, except that no *D. blanci* larvae were found from January to March 2010. Moreover, the maximum length of the larvae in Lake Ozeros was never greater than 300 μm , while in Lake Amvrakia there were several occasions of specimens having lengths greater than 300 μm (reaching up to 350 μm).

Vertical Distribution

The vertical distribution of *D. blanci* larvae was studied along with the spatial distribution in each of the two deeper lakes. In both lakes, greater abundance values of *D. blanci* larvae were recorded in the upper 20 m (Figs. 8, 9) and diminished with depth, especially during the stratification period (May to October). However, the vertical distributions of *D. blanci* larvae differed significantly between the two lakes considering their mean depths (U-test, $p = 0.0001$), with a deeper distribution in Lake Amvrakia. During the stratification period in Amvrakia, the larvae aggregated close to or within the thermocline layer, where chlorophyll-a and oxygen concentrations were higher, while their vertical distribution during the overturn (November to April) was rather uniform (Fig. 9). This pattern was not so obvious in Lake Trichonis, where the larvae mainly seemed to follow the pattern of temperature variation with depth (Fig. 8). It is notable that, despite the general decrease of abundance with increasing depth in Lake Amvrakia, specimens of *D. blanci* larvae were also present in the hypoxic (and sometimes anoxic) deepest layers.

A size-specific depth distribution was noticed in Lake Amvrakia (Figs. 10, 11), with larger larvae residing in deeper strata during the stratification period (Kruskal-Wallis test, $p = 0.0001$). Indeed, the larval size increased with depth until the lower limit of the thermocline (25 m), while below this depth there was no significant difference in larval size (Kruskal-Wallis test, $p = 0.102$). In contrast, during the overturn there was no difference in the size of the larvae under 5 m (Kruskal-Wallis test, $p = 0.273$), whereas the smaller larvae resided in the upper 0–5 m depth (Figs. 10, 11).

Effects of Abiotic Elements

Multiple regression analysis revealed that of the environmental parameters, temperature was the most important influence on the distribution of *D. blanci* larvae in lakes Amvrakia,

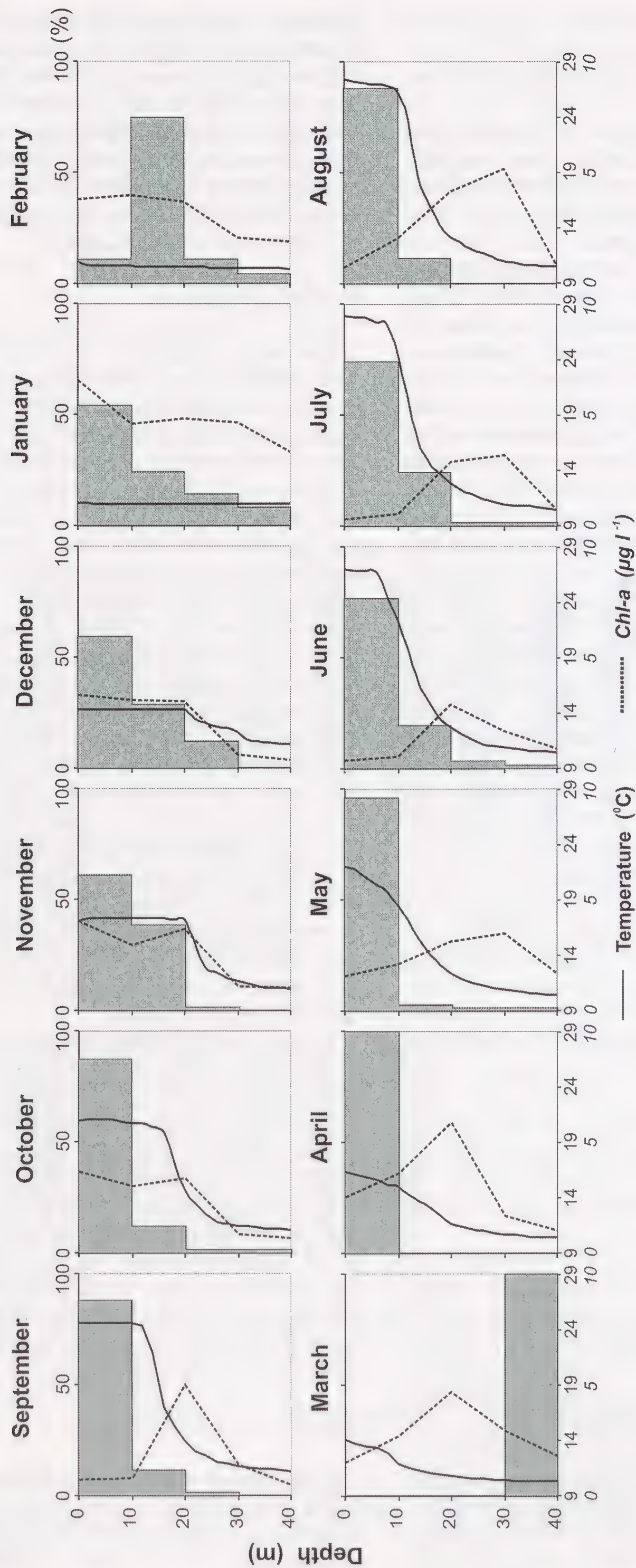


FIG. 8. Vertical profiles of the average temperature (°C) and chlorophyll-a (µg l⁻¹) in Lake Trichonis. The shaded area represents the vertical distribution of *D. blanci* larvae as the average percentage (%) of the total caught during the two-year sampling period. Scales on the x-axis represent temperature (normal) and chlorophyll-a (italics).

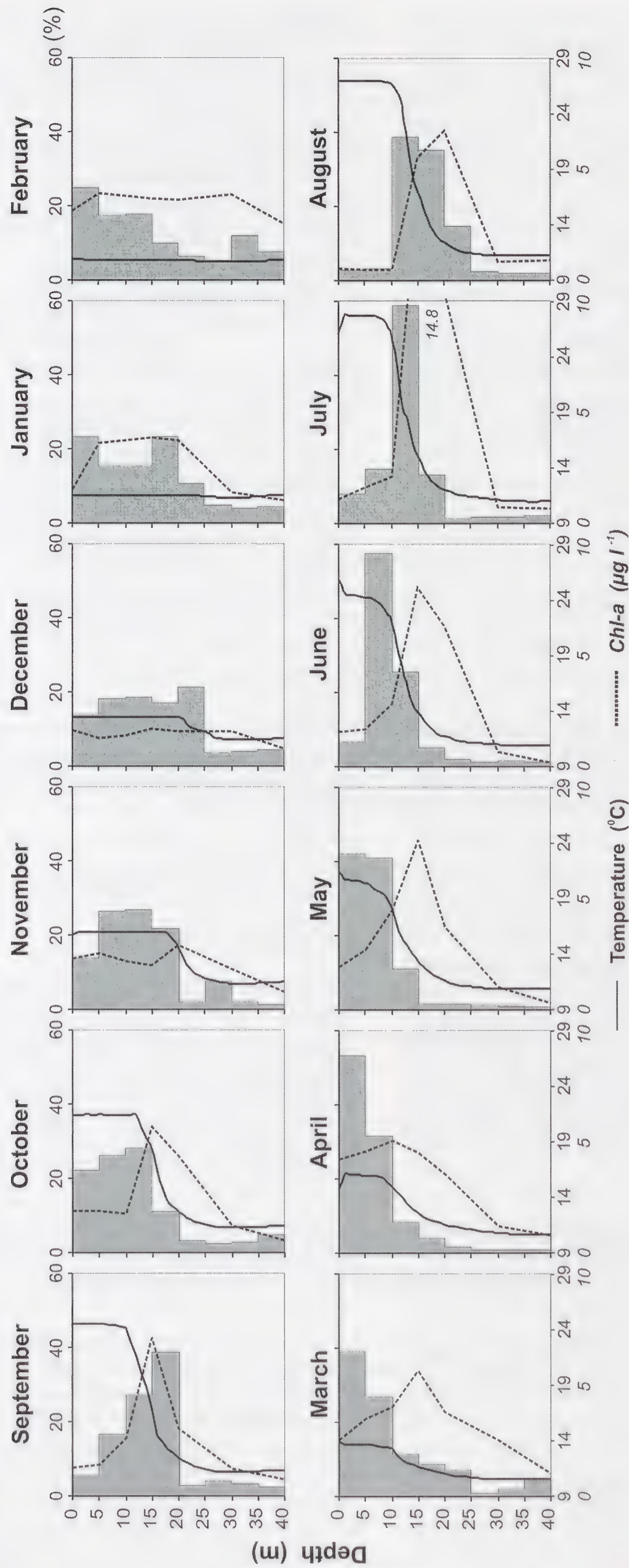
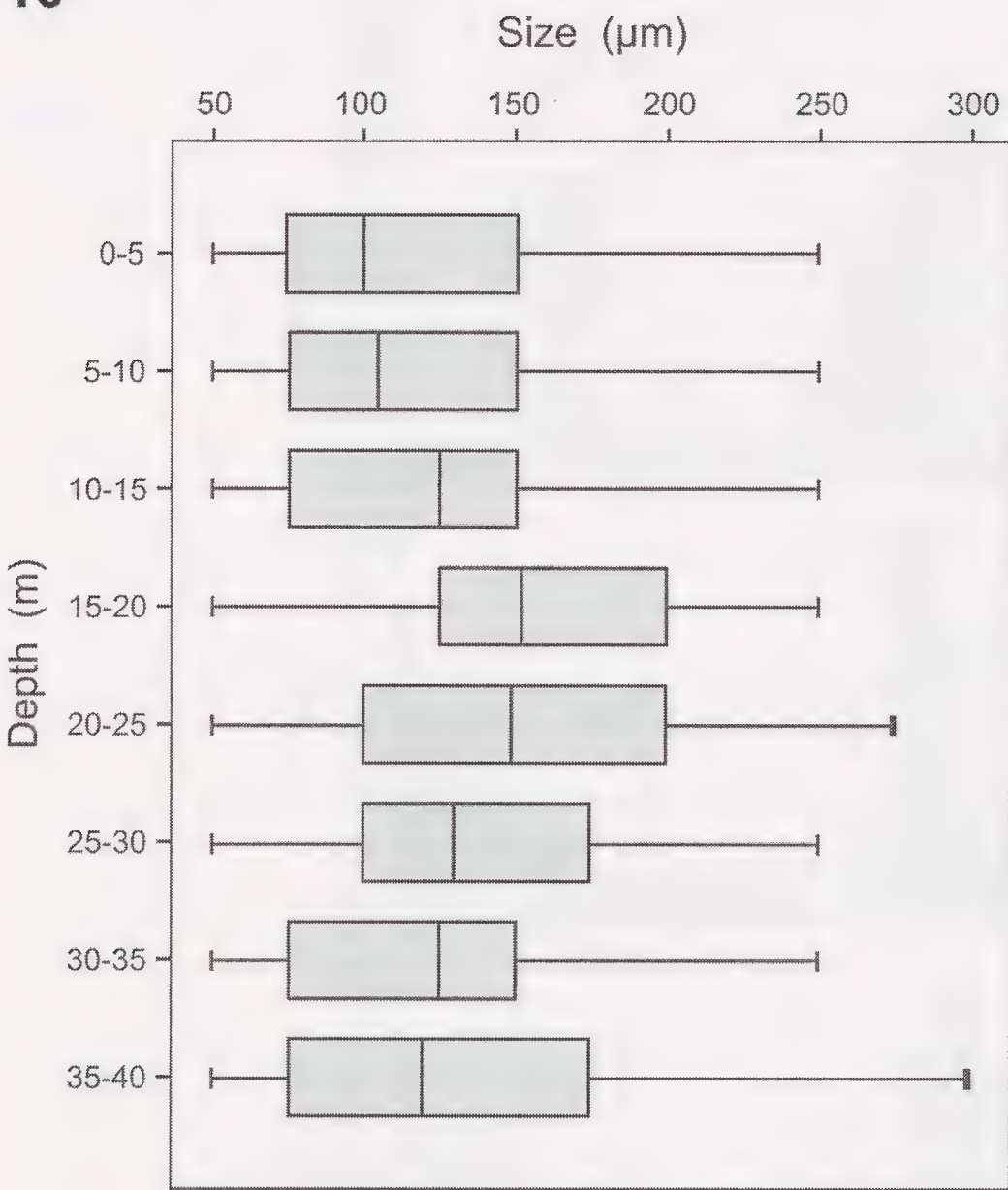
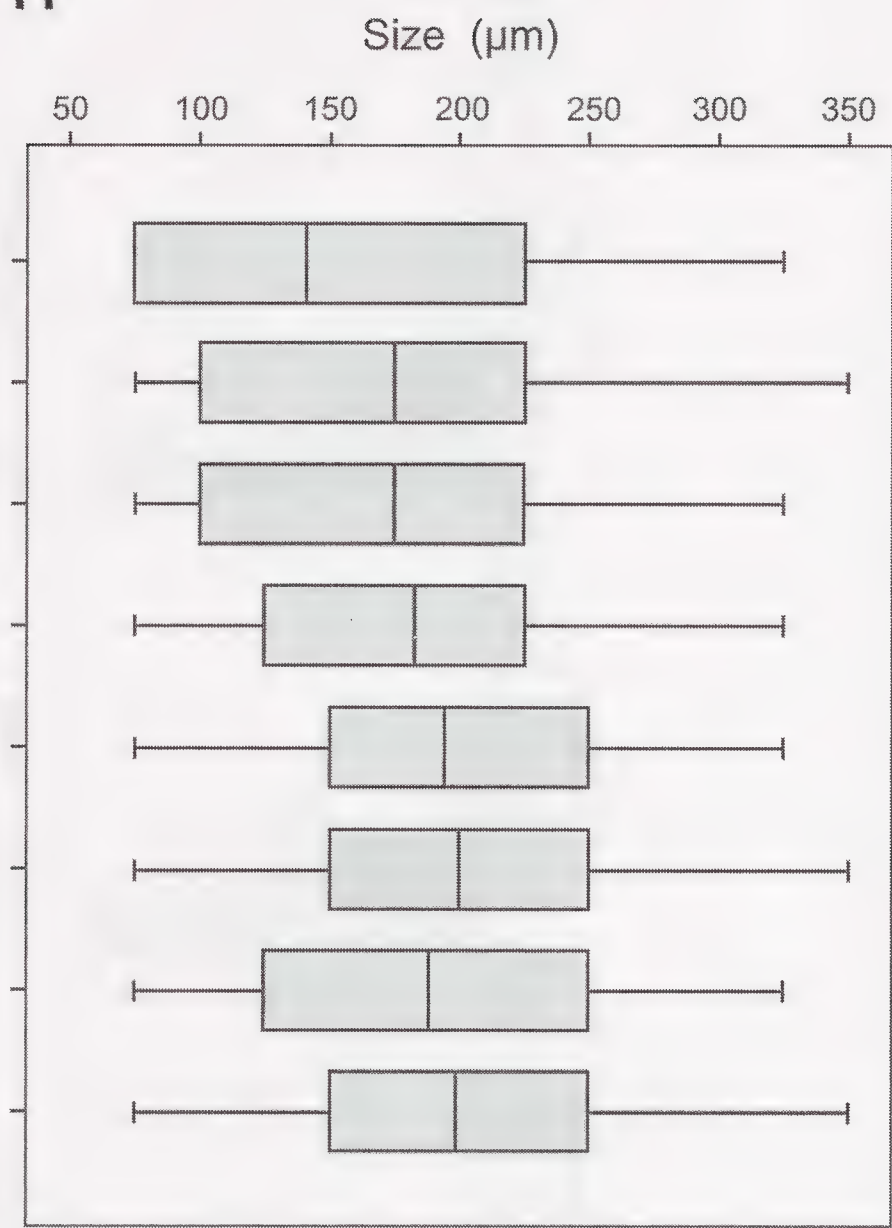


FIG. 9. Vertical profiles of the average temperature ($^{\circ}\text{C}$) and chlorophyll-a ($\mu\text{g l}^{-1}$) in Lake Amvrakia. The shaded area represents the vertical distribution of *D. blanci* larvae as the average percentage (%) of the total caught during the two-year sampling period. Scales on the x-axis represent temperature (normal) and chlorophyll-a (italics).

10



11



FIGS. 10, 11. Size variation of *D. blanci* larvae in the depth intervals sampled. Each boxplot shows the interquartile range of size distribution (box), median (vertical line within the box), and the size range (horizontal lines outside bars). FIG. 10: Size variation during the stratification period (May to October); FIG. 11: Size variation during the turnover period (November to April).

Trichonis and Ozeros. In Stratos reservoir, their distribution was mainly influenced by the water retention time (Table 2). Moreover, the abundance of *D. blanci* larvae was enhanced by conductivity and chlorophyll-a, while transparency seems also to be a significant factor influencing their abundance and distribution. Finally, a significant correlation was found between the larvae abundance variation and water level (WL) in Lake Amvrakia, and with pH in Lake Ozeros.

DISCUSSION

Geographic Distribution

Previous studies on the zooplankton of Lake Trichonis and Stratos reservoir reported the existence of *Dreissena polymorpha* larvae in both freshwater ecosystems (Kehayias et al., 2004; Kehayias et al., 2008; Doulka & Kehayias, 2008, 2011). However, after the recent study of Wilke et al. (2010), who reported the ab-

TABLE 2. Results of the multiple regression analysis between environmental factors like temperature (Temp.), pH, conductivity (Cond.), chlorophyll-a (Chl-a), transparency (Trans.), water retention time (WRT), water level (WR), and the larvae of *Dreissena blanci* in the studied lakes; ** p < 0.01.

Lakes	Temp.	pH	Cond.	Chl-a	Trans.	WRT	WL	r ²	df
Amvrakia	0.633**			0.261**	0.245**	-	0.483**	0.565	143
Trichonis	0.989**					-	-	0.762	51
Ozeros	1.004**	-0.404**				-	-	0.906	11
Stratos						0.707**	-	0.499	15
Total area	0.507**		0.581**	0.139**	-0.200**	-	-	0.550	224

sence of *D. polymorpha* from the area and the presence of *Dreissena blanci* using molecular techniques, the previous findings of the above studies must be reconsidered. According to the present results, *D. blanci* was found in all the five lakes studied, including lakes Ozeros and Lysimachia, for which there were no records in the earlier studies of Albrecht et al. (2007) and Wilke et al. (2010). Consequently, the present investigation supplements the data provided by Albrecht et al. (2007) and Wilke et al. (2010) relative to the geographical distribution of *Dreissena* spp. in the Balkans.

Dreissena blanci is resident in all the lakes of western Greece, including the reservoirs of Acheloos River (Kastraki and Stratos). Apart from Lake Amvrakia, all the other natural lakes (Trichonis, Lysimachia and Ozeros) are connected by natural or man-made channels to the Acheloos River. Its presence in the “isolated” Amvrakia Lake is difficult to explain, unless an accidental transfer of this species from another lake of the area has occurred, e.g. adult mussels attached to boat hulls (Carlton, 1993; Johnson & Carlton, 1996). Another possible explanation for this geographical distribution comes from the geological history of the area. At the end of Pliocene, all the natural lakes of this area (Trichonis, Amvrakia, Lysimachia and Ozeros) formed one large lake (the “Aetoloakarnanian Basin”) that had been created by the Acheloos River outflow. With the gradual lowering of the water level, individual basins became isolated, creating separate lakes (Verginis & Leontaris, 1978). Thus, it may be that *D. blanci* was not transported passively or actively out of Lake Trichonis, but remained as relict populations in these newly isolated lakes.

Although *D. blanci* exists in all sampling sites, there were certain differences in the abundance of its larvae between the five lakes studied. Although these differences might have been due to annual fluctuations, as the samplings were conducted in different years, there could be also other explanations. Surprisingly, the larvae were nearly absent from the zooplankton in Lake Lysimachia, where only a few specimens were found in just a single sampling occasion. Since Lake Lysimachia is connected to and receives water from Lake Trichonis, where a population of *D. blanci* is well established, one would expect a continuous presence of this species in this lake too. Moreover, there are great similarities in the physicochemical characteristics of the water between these two lakes. However, the substantial difference of Lake Lysimachia, not only with Lake Trichonis

but also with the other lakes studied, is its muddy bottom substrate, which is in contrast to the rocky structure of the bottom of the other lakes. Lewandowski (1982b) found that mud and sand are unsuitable substrata for the settlement of the larvae of the relative species *D. polymorpha*. Similarly, it is assumed that the muddy bottom of Lake Lysimachia could be the restricting factor for the existence of *D. blanci* larvae in this area. In addition, several samplings conducted in the canal connecting Lysimachia and Trichonis lakes always revealed specimens of *D. blanci* larvae, meaning that there was a constant “inflow” of larvae from Trichonis. Consequently, it is suggested that most, or probably all, the larvae that enter Lysimachia from Trichonis fail to settle in the former because due to the unsuitable substratum, they are not able to form a viable population. In fact, it is possible that the few larvae found in Lake Lysimachia on only one occasion, could have been specimens just recently transferred from Trichonis Lake. On the other hand, differences in the abundance of *D. blanci* larvae between the two deep lakes Trichonis and Amvrakia could be due to their trophic status, as Trichonis is considered oligo- to mesotrophic (Doulka & Kehayias, 2008), whereas Amvrakia is mesotrophic (Overbeck et al., 1982; Chalkia et al., 2012). Thus, the higher abundances of *D. blanci* larvae in Lake Amvrakia may have been due to the higher presence of food here.

Seasonal Variation

The seasonal variation of *D. blanci* larvae was characterized by their presence in the zooplankton for most of, or for the entire year. This is also noticed for *D. polymorpha* according to Lewandowski (1982a), who stated that the duration of the planktonic phase of its larvae differs among lakes and, generally, increases from north to south in the Balkan lakes as a result of temperature. However, there have been some cases where over-wintering larvae of *D. polymorpha* have been found in the plankton even at temperatures as low as 7.2°C (Lucy, 2006). This could also explain the presence of *D. blanci* larvae in Stratos reservoir during February 2005 and 2006, when temperatures were lower than 9°C. Increasing water temperature during spring has been widely reported as the primer environmental trigger for the reproduction of *D. polymorpha* when surface temperatures rise above 12°C (Sprung, 1989; Borchering, 1991), or above 15°C (Einsle, 1973; Stańczykowska, 1977; Karatayev et al.,

1998). Similarly, the first appearance of the smallest (S1) larvae of *D. blanci* after the winter period was in March (Lake Amvrakia), or April (Lake Ozeros), when the surface temperature reached 13.5°C and 19°C, respectively. Moreover, the influence of temperature on the seasonal variation of *D. blanci* larvae was reflected by the characteristic increase of abundance during the warmer period of the year, starting in spring, while there was a decrease in autumn and winter. This is in agreement with reports on the seasonal abundance variation of *D. polymorpha* from other lakes of Europe (Borcherding, 1991; Sprung, 1993), the northern Balkans (Stanković, 1960; Lewandowski, 1982a) and northern Greece (Michaloudi et al., 1997; Zarfdjian et al., 1990).

Considering the seasonal size distribution of *D. blanci* larvae with their abundance variation in lakes Amvrakia and Ozeros, it is suggested that the reproductive period of this species starts in spring and continues until late autumn. Based on reports on the life cycle of the related *D. polymorpha*, we assumed that larvae smaller than 100 µm (S1) were probably at the stage of entering the planktotrophic period (Sprung, 1993). These larvae must have been the offspring of an earlier reproduction and, consequently, their presence in almost all sampling months indicates the existence of several reproductive incidents, or continuous reproduction. S2 larvae (100–200 µm in length) could correspond to those in a pre-settling stage, while S3 larvae (200–300 µm) are probably at the settling stage. The maximum length of the larvae in Lake Ozeros was lower compared to Lake Amvrakia, where several specimens reached lengths up to 350 µm. According to Sprung (1993), larger larvae may be the result of unsuitable substrata, causing the larvae to delay their metamorphosis. Conditions with negative impacts on settling success of *D. polymorpha* larvae include unfavorable oxygen concentrations in the hypolimnion and unsuitable bottom structures (Stańczykowska, 1977), while the depth at which the larvae generally settle is usually not greater than 10 m (Sprung, 1993). The oxygen content in Ozeros Lake was sufficient during the entire sampling period, while there were no hypoxic or anoxic conditions within the water column in any case. In contrast, hypoxic (dissolved oxygen < 2 mg l⁻¹) or even anoxic (dissolved oxygen < 0.2 mg l⁻¹) conditions were recorded in Lake Amvrakia in the hypolimnetic layer and especially under 30 m during the stratification period. In addition, the greater depth of

the sampling sites of Lake Amvrakia (over 20 m) could have been responsible for a lower settling success and consequently to the occurrence of larger larvae in the zooplankton samples.

Vertical Distribution

Investigation of the vertical distribution of *D. blanci* larvae in the two deeper lakes of western Greece (Trichonis and Amvrakia) showed that most of the specimens inhabited the epilimnetic layer. This general trend was especially recorded during the stratification period (May–October), in contrast to their nearly uniform distribution during the overturn period (November–April). Similarly, studies on the vertical distribution of the relative species *D. polymorpha*, reported maximum abundance within the epilimnion during the stratification period, and a uniform vertical dispersion along the water column during the unstratified period (Sprung, 1993; Lewandowski & Eijsmont-Karabin, 1983).

Although there were certain similarities in the vertical distribution patterns of the larvae between the lakes Trichonis and Amvrakia, differences were also noticed resulting in a general trend of deeper distribution in Amvrakia. On several occasions, mainly during the stratification period, the larvae in Lake Amvrakia were aggregated to the metalimnion where maximum chlorophyll-a was observed. Indeed, this trend was verified by the significant correlation between chlorophyll-a and larval abundance in the vertical axis. As chlorophyll-a was used to estimate primary production in Amvrakia Lake, there seems to be an interrelation of the larvae to the phytoplankton concentration, which, in turn, could suggest a kind of dependence on specific food elements. The verification of this hypothesis would require larvae diet analysis, as well as data on the abundance, distribution and composition of phytoplankton in Lake Amvrakia.

Thermal stratification in both lakes Trichonis and Amvrakia played a crucial role on the vertical distribution of the larvae. It has been well documented that stratification results in the existence of optimum epilimnetic environmental factors (temperature, pH and dissolved oxygen) for *D. polymorpha* compared to those prevailing in the hypolimnion (Sprung, 1987; Hincks & Mackie, 1997; Karatayev et al., 1998; Gelda et al., 2001). In Lake Trichonis there was little variation in pH values with increasing depth, with an average value of 8.11. In Lake Amvrakia,

however, the pH value for the entire water column was lower than Lake Trichonis (7.62) and there was a sharp decrease in the hypolimnion (7.31), especially during stratification (7.23). In addition, a strong positive correlation (Pearson's $r = 0.40$, $p < 0.001$) was found between pH values and larval abundance in the vertical axis. Consequently, this parameter could have significant influence on the vertical distribution of *D. blanci* larvae in Lake Amvrakia.

Oxygen concentration is considered among the most important abiotic parameters affecting zooplankton along the vertical axis of aquatic ecosystems, since most of these organisms tend to avoid living in hypoxic or anoxic conditions and remain restricted to the well oxygenated parts of the water column (Żurek, 2006; Vanderploeg et al., 2009, and references therein). The oxygen concentrations in the epilimnion and especially in the metalimnion of Lake Amvrakia were always high and, thus, sufficient for larvae growth and survival. However, in the hypolimnion the oxygen concentrations in certain periods were below 2 mg l^{-1} , which is a known critical level for *D. polymorpha* growth (Mihuc et al., 1999). Surprisingly, a considerable number of specimens of *D. blanci* larvae were recorded in these hypoxic parts of the lake and even in the deeper layer (35–40 m), which was anoxic at the end of stratification period. It has been documented that freshwater mussels such as *D. polymorpha*, are able to survive short-term exposure (< 5 days) to anoxic or hypoxic conditions in their habitat (Johnson & McMahon, 1998). In addition, some crustacean species can survive in hypoxic and even anoxic conditions for a small period of time using hypoxic zones as a potential refuge against fish predation during daylight, while they migrate to shallower depths at night (Vanderploeg et al., 2009). Doulka & Kehayias (2011), investigating the diel vertical migration (DVM) of zooplankton taxa in Lake Trichonis, found that *D. blanci* larvae (misidentified as *D. polymorpha*) were able to perform vertical migrations and ascend to shallower depths during the night. This ethological adaptation could be a possible explanation for the presence of larvae in such unfavorable conditions during the day, although there is lack of evidence for DVM in Lake Amvrakia as samplings were conducted only in morning hours.

An interesting feature of *D. blanci* larvae was their size-specific depth distribution. During stratification, an increase of their size up to the lower limit of the metalimnion was observed,

while this phenomenon was not recorded during the unstratified period. This means that stratification was probably responsible for or enhanced this particular pattern of vertical distribution. There could be several explanations for this. One could assume a passive sinking of the larger larvae, which probably have higher specific weight, until they reach the metalimnion, where the increased water density balances their descent. Also, considering an energetic movement of the larvae, the presence of larger specimens in the metalimnetic layer could be associated with the higher phytoplankton concentration, taking into account the high chlorophyll-a concentrations recorded in this layer. The reduction of intra-specific food competition may also be another mechanism of size separation of zooplankton in the vertical axis (Doulka & Kehayias, 2008, 2011). Finally, the selection of greater depth by the larger larvae could reduce their predation by planktivorous fish foraging in the illuminated epilimnetic waters (De Meester et al., 1999).

Effects of Abiotic Elements

As previously discussed, temperature is clearly the decisive factor in the life cycle of other dreissenids. Thus, the present results on *D. blanci* larvae are in accordance with reports about the positive relation of temperature on the abundance variations of *D. polymorpha* larvae in lakes of Poland (Stańczykowska & Lewandowski, 1993) and Mikri Prespa Lake (Michaloudi et al., 1997). The influence of temperature may be considered as direct, controlling the length of time that the larvae are found in the plankton and affecting the growth of adult specimens (Sprung, 1993; Karatayev et al., 2006), or indirect due to increased food availability (Mantecca et al., 2003).

The correlation between phytoplankton biomass (as reflected by the chlorophyll-a concentration) and the abundance of *D. blanci* larvae in Lake Amvrakia, was due mainly to similarities in their vertical distribution than to their seasonal variation. In any case, it is expected that food quality rather than quantity is most important for growth and reproduction of *D. blanci* larvae, as found also for the related *D. polymorpha* (Stoeckmann & Garton, 2001).

The negative interrelation of pH with larvae variation in Lake Ozeros is contradictory to the positive interrelation of this element in Lake Amvrakia, as previously discussed. To our knowledge, there is not much data on this

issue, except the reference of Hincks & Mackie (1997), who reported that positive growth of *D. polymorpha* larvae only occurred at pH levels greater than 8.3.

Water level (WL) measurements, which were conducted only in Lake Amvrakia, showed differences between the two years of study, while this variation turned to have some considerable effects in the abundance of *D. blanci* larvae. Water level fluctuation is expected to exercise several indirect influences affecting the total nutrient load, phytoplankton production and consequently the chlorophyll-a concentration within the lake. On the other hand, the decrease of larval abundance in the second year could have been a direct effect of water level reduction, which uncovered an extensive area of the bottom of the lake, being the habitat for the adult forms of this mollusk. Therefore, it can be assumed that this habitat reduction probably resulted in the reduced reproductive capacity of the population.

Water retention time (WRT) was estimated only in Stratos reservoir for a two-year period and varied between 2.3 to 10.8 days (Kehayias et al., 2008), and presented significant differences throughout the two-year study. WRT proved to be the most significant parameter in this reservoir, exercising a positive effect on the abundance variation of *D. blanci* larvae. Stratos reservoir receives water from the upstream Kremasta reservoir, which is also inhabited by *D. blanci* larvae (reported as *D. polymorpha* by Koussouris et al., 1994). In the Kremasta reservoir, however, this species was by far dominant in the zooplankton, in contrast to Stratos. The main difference in these two reservoirs lies in their WRT, which for Kremasta is nearly a year, in contrast to a few days for Stratos. Thus, it could be assumed that *D. blanci* is probably better adapted to the higher stability of the lacustrine zone in Kremasta reservoir, where it can form larger populations. This could also explain the decrease of its abundance during the second sampling year, when the water outflow into Stratos reservoir was greater, resulting in even lower WRT values. However, several reports point out that the attachment of *D. polymorpha* larvae can be facilitated by high water flow, which probably increases the frequency of contact between organisms and substrate (Navarro et al., 2006).

In conclusion, the present results reveal that there are great biological and ecological similarities between *D. blanci* and *D. polymorpha* larvae. This could be of great importance

when taking into account the notorious invasive character of the sister species *D. polymorpha* and its various impacts on freshwater ecosystems (Minchin et al., 2002). In this sense, there is a potential threat that *D. blanci* could become another invasive species in freshwater ecosystems and therefore appropriate management measures would be required also for this species. Moreover, similar to its sister species (*D. polymorpha*), *D. blanci* could cause certain economic effects, such as the fouling of intake pipes and drinking water pipelines, as in the case of Kremasta and Stratos reservoirs (unpublished data). Consequently, the present study can be considered as a first step in the investigation of the biology and ecology of *D. blanci*, however considerably more research is needed on the life cycle of this species, and the ecological and economic consequences of its inhabitation in lakes and reservoirs of this area of Europe.

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VARIATION OF SNAIL ASSEMBLAGES IN HAY MEADOWS: DISENTANGLING THE PREDICTIVE POWER OF ABIOTIC ENVIRONMENT AND VEGETATION

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ABSTRACT

We studied snail assemblages at 39 plots in hay meadows of the White Carpathians, Czech Republic. We recorded snails quantitatively in 1 m² quadrats, along with vegetation and several measured variables in the same plots in order to investigate the influence of selected environmental factors on meadow snail species composition and richness. The study aimed to determine the extent to which it is possible to use vegetation as a predictor of land snail composition by comparing the predictive power of three groups of variables: (i) measured variables and climatic factors, (ii) vegetation, and (iii) Ellenberg indicator values for plants estimated from plant composition.

Detrended correspondence analysis revealed that both snail and plant assemblages were strongly affected by the main environmental gradient running from calcium-poor, wet and cool upland sites to calcium-rich, dry and warm lowland sites. The main changes of vegetation and snail species composition were highly correlated ($r_s = 0.77$, $p < 0.001$). Soil calcium content and moisture were the most important factors, which explained most of the variation both in the snail and plant assemblages. The interaction of these two factors was even stronger but it was not possible to separate the influence of particular variables on snail species composition and to determine which of these variables made the greatest contribution to the observed pattern. Using the variance partitioning approach, we found that most variation in snail species data was jointly explained by all three groups of variables (i.e., measured variables and climatic factors, vegetation, and Ellenberg indicator values for plants). Even so, the net effects of both vegetation and measured variables were significant. This indicates that vegetation could describe important environmental controls of land snail distribution, even those that are difficult or even impossible to measure directly. We conclude that vegetation composition can be very useful predictor in snail community ecology studies.

Key words: gastropods, vegetation, Ellenberg indicator values, grasslands, White Carpathians.

INTRODUCTION

Hay meadows of the White Carpathian Mountains are among the most extensive and species rich grasslands in Europe (Jongepierová, 2008). In general, little work has been done on hay-meadow snail assemblages, which is probably due to low snail species richness in meadow habitats. Most snail species of temperate zone inhabit forests, which offer higher microhabitat heterogeneity (Ložek, 1956), stable micro- and mesoclimatic conditions (Martin & Sommer, 2004a) and available shelters to survive droughts and low temperatures. In forests, snails inhabit not only topsoil, but also standing woody stumps and decaying wood, cavities un-

der tree bark and leaf litter, thus they are not so closely related to soil substratum (e.g., Ložek, 1962). Except the Mediterranean region, European mollusc faunas include a limited number of species adapted to dry and open habitats (Ložek, 1964). Steppe species associated with open habitats are closely related to soil conditions and mostly require limestone formations or other calcium-rich substratum (Ložek, 1962). In contrast, Central European hay meadows are human-maintained habitats that originated after deforestation since the Neolithic (Cameron & Morgan-Huws, 1975; Chappell et al., 1971; Young & Evans, 1991). These meadows often occur on deep, originally forest soils that have lost calcium carbonate due to leaching.

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Soil calcium content and pH are in most cases considered as limiting factors that determine terrestrial gastropod distributions at various spatial scales (Burch, 1955; Horsák, 2006; Horsák & Hájek, 2003; Juříčková et al., 2008; Ložek, 1962; Valovirta, 1968; Waldén, 1981). Snails need calcium for the growth of their shells (Wärebörn, 1970) and for their successful reproduction (Wärebörn, 1979). The importance of calcium was observed also in meadow habitats where aluminum and magnesium content were also found to be important (Ondina et al., 1998, 2004). Soil texture is another important factor influencing meadow snail assemblage composition (Hermida et al., 1995; Ondina et al., 2004; Outeiro et al., 1993), along with moisture and vegetation structure (Davies & Grimes, 1999; Martin & Sommer, 2004a). All of these variables are often interrelated, and thus it is difficult to define precisely the main factor that determines land snail distribution. Especially vegetation structure and composition of meadow habitats is closely connected with land management (Cameron & Morgan-Huws, 1975; Jongepierová, 2008). The impact of different management practices and vegetation succession on snail assemblages was observed in several studies (Baur et al., 2006; Boschi & Baur, 2008; Cremene et al., 2005; Labaune & Magnin, 2002; Ruesink, 1995). In general, vegetation structure was found to be more important explanatory factor than floristic composition because microclimate conditions are more related to vegetation structure than to plant species composition (Labaune & Magnin, 2001). Other studies showed that vegetation composition was more important for explaining variation in snail species of spring fens than water chemistry (Horsák & Hájek, 2003; Horsák et al., 2011). It was also shown that plant indicator values (Ellenberg et al., 1992) were a useful tool for land mollusc autecology assessment (Horsák et al., 2007b).

Thus, it is not surprising that the gross distribution of both snails and plants relate to the same environmental gradients. The questions remain as to possible differences at small scales, and the extent to which explanatory variables based on plant species composition can act as surrogates for others that are harder or more expensive to obtain. Therefore, we recorded both snails and plants inhabiting the same plot in the same time. Further, for the explanation of variation in snail assemblages we not only used several directly measured factors but also variables obtained from vegetation composition.

We established three main aims of this study: (i) to investigate the influence of selected environmental factors on snail species composition and richness in hay meadows, (ii) to determine to what extent it is possible to use vegetation as a predictor of land snail composition by comparing the predictive power of three groups of variables: measured variables and climatic factors, vegetation, and Ellenberg indicator values for plants, and (iii) to compare effects of the studied factors between snail and plant assemblages.

MATERIAL AND METHODS

A total of 39 plots distributed in 27 localities were studied in dry grasslands and mesic meadows in the White Carpathians Mountains, southeastern Czech Republic (Fig. 1) in 2005–2008. The extent of the study area is 715 km². Geologically it belongs to the outer part of the Western Carpathians, mostly to the Magura Flysch. This is bedrock formed by Cretaceous and Palaeogene sea sediments consisting of alternating sandstone and claystone layers of variable thickness (Jongepierová, 2008). These sediments often contain a considerable amount of calcium carbonate, but most of the studied meadows have developed on originally forest soils and have a secondarily decalcified topsoil layer. Exceptions are small patches with shallow soils around the outcrops of calcareous bedrock. There are also climatic and historical differences within the study area. Altitude and precipitation increase and temperature decreases towards the northeast. The studied localities are at altitudes 296–667 m and have average annual precipitation amounts of 555–907 mm and average annual temperatures of 6.8–8.5°C. Geographical influences and altitude are closely connected with historical development of the area. Meadows at higher altitudes, especially in the northeastern part of the study area, have originated later than meadows in lowlands in the southwest due to the different timing of human settlement (Jongepierová, 2008). The vegetation of these meadows has been classified into three phytosociological alliances (Jongepierová, 2008): (i) the subcontinental broad-leaved dry grasslands of *Cirsio-Brachypodium pinnati* Hadač et Klika ex Klika, 1951, (ii) subatlantic broad-leaved dry grasslands of *Bromion erecti* Koch, 1926, and (iii) mesic meadows of *Arrhenatherion elatioris* Luquet, 1926.

All studied meadows are managed by mowing twice a year, only exceptionally once a year

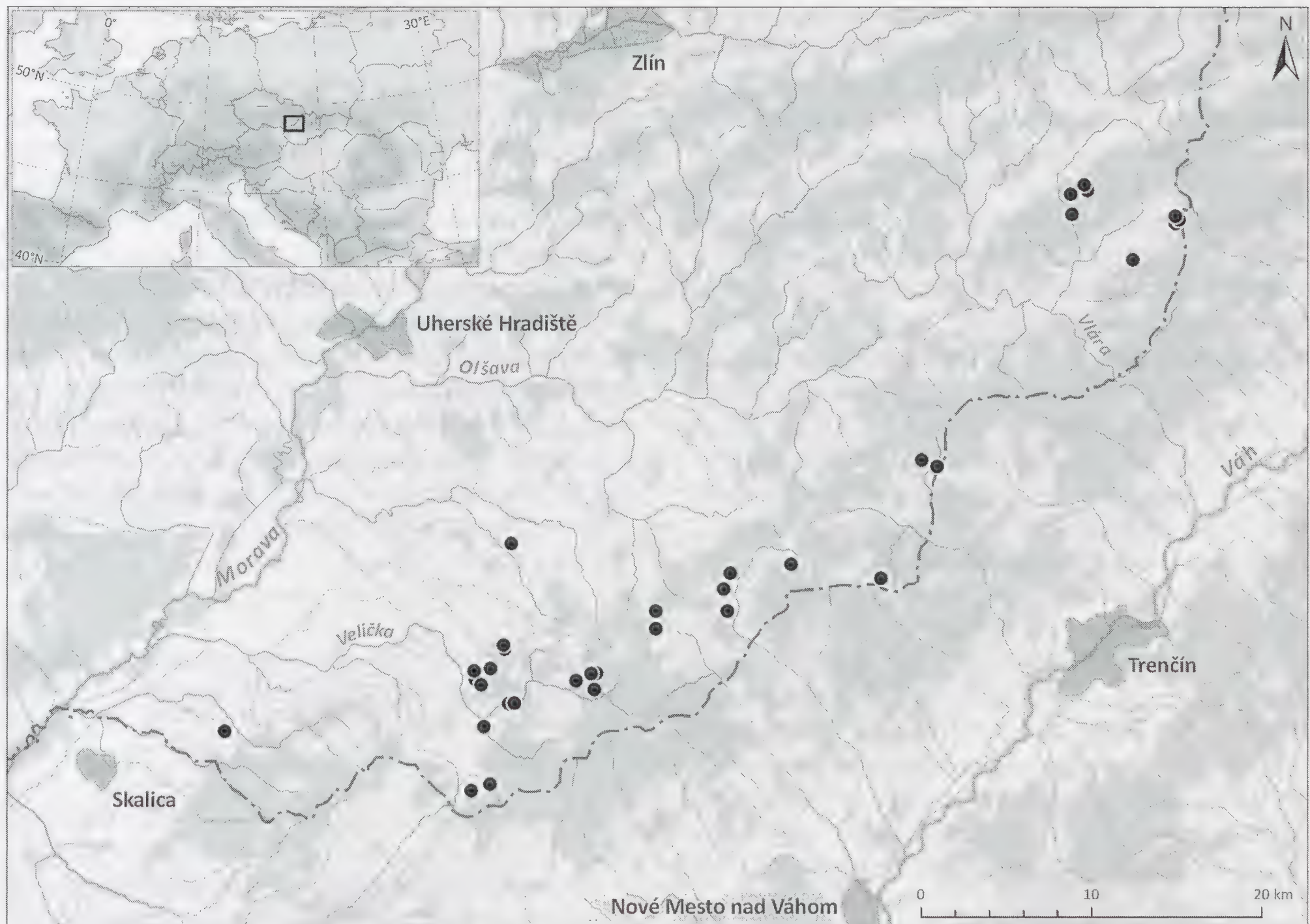


FIG. 1. Map of the studied area with 39 study sites marked by points.

in the case of driest meadows with sparse vegetation. All plots of 1 m² were sampled in spring, mostly at separated meadow sites in order to avoid pseudoreplications. In each plot, cover of all vascular plant species was recorded on the nine-grade Braun-Blanquet scale (Westhoff & van der Maarel, 1978). Then, in each plot, the upper soil layer up to the depth of 5 cm was taken from four randomly chosen 25 x 25 cm quadrats for quantitative sampling of snail assemblages. After snail sampling, topsoil samples were collected from each plot for laboratory measurements of soil calcium content and pH. A 2:5 soil-distilled water solution was prepared from each sample and measured after 20 h by a pH-meter. A 1:10 Mehlich III solution was filtered after ten minutes of shaking, and soil calcium content was measured by an atomic absorbing spectrophotometer in an accredited laboratory. The snail samples were dried and then extracted using standard procedures and separated by hand-sorting under a binocular microscope (Kerney et al., 1983; Ložek, 1956). Live snails and empty shells were counted separately. Counts from four quadrats

of each 1 m² plot were merged together. Soil moisture was measured in the field in October 2008 by one-shot measurement at each plot during three days of stable dry weather without precipitation. We used Theta Probe ML2x hygrometer, which measures moisture of the topsoil layer up to the depth of 10 cm.

In total, we used 22 explanatory variables which were divided into three groups: (i) Group I, which includes measured soil variables (soil pH, soil calcium content and soil moisture), further basic climatic factors (mean annual precipitation and mean annual temperatures) obtained from digital maps of climatic factors (Tolasz, 2007) by overlying them in the geographic information systems (ESRI, 2003), topographic indices of solar radiation and heat load (radiation, heat) calculated from the slope, aspect and geographic coordinates (McCune & Keon, 2002), altitude, latitude and longitude; (ii) Group II, consisting of variables obtained based on Ellenberg's indicator system (EIVs) used in vegetation science (e.g., Ertsen et al., 1998; Ewald, 2003; Hill & Carey, 1997; Schaffers & Sýkora, 2000; Wamelink et al., 2002).

EIVs are empiric values assigned to each of 2726 Central European vascular plant species to reflect their ecological behaviour. EIVs express, on an ordinal scale, the relationship of plant species to chosen ecological gradients (E-light, E-temperature, E-continentality, E-moisture, E-reaction, and E-nutrients). This scale is 12-point for moisture and a 9-point for the other variables (Ellenberg et al., 1992). The value for each plot was calculated as an average of all values of each plant species recorded in the plot; (iii) Group III, vegetation; variables expressing vegetation structure (the cover of herbs and mosses) and plant species composition expressed as the ordination site scores on the first four DCA (Detrended correspondence analysis) axes (V1DCA–V4DCA; see below).

The relationships among explanatory variables were summarized using principal component analysis (PCA) on a correlation matrix calculated using STATISTICA 7 (Hill & Lewicki, 2007). The snail data were adjusted before statistical analyses. All slugs (two species) were omitted because our sampling method did not allow accurate assessment of their occurrence and abundance (Oggier et al., 1998). For multivariate analyses, we used numbers of live individuals, because abundance of empty shells is strongly influenced by levels of soil calcium (Cernohorsky et al., 2010; Claassen, 1998) and can be also linked with decay time of shells of particular species (Ménez, 2002; Pearce, 2008). We also used in the analyses species with no live individual recorded in the particular site, but only those where at least one freshly dead shell (i.e., with an intact periostracum) was recorded. Therefore, we added 1 to the number of live individuals of each recorded species; the abundance of species recorded only as fresh empty shell(s) was equal to 1. This allowed to capture also low-abundant living species, which were very probably present at studied plots, but not recorded as live by sampling. Abundances were log-transformed as $Y = \log(n + 1)$ to undo the influence of dominant species. Rare species were down-weighted as recommended for DCA (Lepš & Šmilauer, 2003). For multivariate analyses of vegetation, we used untransformed estimates of cover.

The species-by-plot matrices for snail and plant samples were separately subjected to DCA. The ordination site scores obtained were correlated with each other and with explanatory variables using the Spearman correlation coefficient. Canonical correspondence analysis (CCA) and the variation partitioning approach (Borcard et al., 1992) were used to segregate variation of snail

data among three groups of variables (measured variables, EIVs, vegetation). In the first step, we used CCA and a forward selection method to isolate three best predictors out of each group because explained variation is also influenced by the number of explanatory variables (Peres-Neto et al., 2006). These variables explained most variation within snail assemblages. In the next step, we used partial CCAs and the variation partitioning approach to segregate variation among these nine variables. Significance of the canonical axes was testing by Monte Carlo test with 1,999 permutations. The multivariate analyses were done using the CANOCO 5 package (ter Braak & Šmilauer, 2002). Spearman correlation coefficient was calculated in STATISTICA 7 (Hill & Lewicki, 2007).

TABLE 1. Coordinates of 22 explanatory variables on the first two axes of PCA on correlation matrix of 39 plots. Significance is given as: n.s. - not significant; * - $p < 0.05$; ** - $p < 0.01$.

Explanatory variables	1st PCA axis	2nd PCA axis
Eigenvalue	8.316	3.621
Percentage variance explained	37.8	16.46
Latitude	n.s.	0.81**
Longitude	-0.51*	0.79**
Soil pH	0.70**	n.s.
Soil calcium content	0.78**	n.s.
Soil moisture	-0.46*	-0.47*
Altitude	-0.77**	0.33*
Mean annual temperature	0.75**	-0.51*
Mean annual precipitation	-0.78**	0.34*
Radiation	0.43*	0.52*
Heat	0.42*	0.49*
E-light	0.62**	0.45*
E-temperature	0.70**	n.s.
E-continentality	0.70**	n.s.
E-moisture	-0.77**	n.s.
E-reaction	0.84**	n.s.
E-nutrients	-0.63**	-0.52*
V1DCA	-0.91**	n.s.
V2DCA	n.s.	n.s.
V3DCA	n.s.	n.s.
V4DCA	n.s.	n.s.
Moss cover	-0.33*	n.s.
Herb cover	-0.64**	n.s.

RESULTS

Table 1 shows the relationships of the explanatory variables to the first and second PCA axes; however, most of the measured explanatory variables were closely interrelated. PCA detected a strong environmental gradient associated with the first axis, running from calcium poor, wet, cold and upland sites to the calcium rich, dry, warm and lowland sites with lower precipitation (Table 1). The second PCA axis reflected the geographical gradient from the northeastern to southwestern part of the White Carpathian Mountains. The third and higher axes explained a very small part of the total variation and their correlations with individual variables were not significant.

The studied snail assemblages were rather poor in species. In total, we found 29 snail species and collected 5,285 live individuals and 13,662 empty shells (Table 2). On average 5 (min. 4, max. 10) live snail species and 117 (min. 13, max. 621) live individuals per plot were collected. Numbers of species as well as numbers of individuals were strongly correlated with soil calcium content, pH and E-reaction (Table 3). We observed a higher predictive power of the interaction between measures of calcium and site moisture.

Snail species composition and its relation to explanatory variables were studied using DCA. We obtained only one strong gradient of snail species composition, which was mainly associated with E-moisture ($r_s = 0.75$; $p < 0.001$),

TABLE 2. List of all analysed species, frequency of live and all individuals at study 39 sites, number of all live individuals, and number of all empty shells.

Species	Freq. of live individuals	Freq. of all individuals	No. of live individuals	No. of empty shells
<i>Punctum pygmaeum</i> (Draparnaud, 1801)	38	39	1048	2715
<i>Vertigo pygmaea</i> (Draparnaud, 1801)	31	35	962	2607
<i>Cochlicopa lubricella</i> (Rossmässler, 1838)	27	31	398	762
<i>Vallonia pulchella</i> (Müller, 1774)	24	29	562	1273
<i>Vitrina pellucida</i> (Müller, 1774)	21	24	509	513
<i>Perpolita hammonis</i> (Ström, 1765)	21	27	166	349
<i>Truncatellina cylindrica</i> (Férussac, 1807)	19	21	1177	2903
<i>Euomphalia strigella</i> (Draparnaud, 1801)	16	29	73	110
<i>Granaria frumentum</i> (Draparnaud, 1801)	7	8	22	1105
<i>Acanthinula aculeata</i> (Müller, 1774)	7	11	48	210
<i>Cochlicopa lubrica</i> (Müller, 1774)	6	9	39	65
<i>Vallonia costata</i> (Müller, 1774)	5	9	167	255
<i>Aegopinella minor</i> (Stabile, 1864)	5	14	30	419
<i>Vitrea contracta</i> (Westerlund, 1871)	2	8	6	84
<i>Euconulus fulvus</i> (Müller, 1774)	1	4	1	6
<i>Cepaea vindobonensis</i> (Férussac, 1821)	1	4	5	35
<i>Ceciloides acicula</i> (Müller, 1774)	1	2	19	170
<i>Oxychilus inopinatus</i> (Uličný, 1887)	1	1	5	4
<i>Carychium tridentatum</i> (Risso, 1826)	1	2	1	25
<i>Vertigo angustior</i> Jeffreys, 1830	1	3	43	35
<i>Petasina unidentata</i> (Draparnaud, 1805)	1	1	2	1
<i>Pupilla muscorum</i> (Linnaeus, 1758)	0	4	0	7
<i>Carychium minimum</i> Müller, 1774	0	2	0	2
<i>Alinda biplicata</i> (Montagu, 1803)	0	2	0	2
<i>Aegopinella pura</i> (Alder, 1830)	0	1	0	1
<i>Vertigo pusilla</i> Müller, 1774	0	1	0	3
<i>Vitrea diaphana</i> (Studer, 1820)	0	1	0	1

TABLE 3. Spearman correlations among species data and explanatory variables (including their interactions - x). Significance is given as: n.s. - not significant; * - $p < 0.05$; ** - $p < 0.01$.

Explanatory variables	No. of live individuals	No. of live species	No. of all individuals	No. of all species
Soil pH	n.s.	n.s.	0.51*	0.39*
Soil calcium content	0.45*	n.s.	0.66**	0.46*
Soil moisture	n.s.	n.s.	n.s.	n.s.
Altitude	n.s.	n.s.	-0.40*	-0.43*
Mean annual precipitation	n.s.	n.s.	n.s.	n.s.
Mean annual temperature	n.s.	n.s.	0.37*	0.34*
Radiation	n.s.	n.s.	n.s.	n.s.
Heat	n.s.	n.s.	0.33*	n.s.
E-light	n.s.	n.s.	0.36*	n.s.
E-temperature	n.s.	n.s.	0.33*	n.s.
E-continentality	n.s.	n.s.	n.s.	n.s.
E-moisture	n.s.	n.s.	n.s.	n.s.
E-reaction	n.s.	n.s.	0.48*	0.41*
E-nutrients	n.s.	0.41*	n.s.	n.s.
Soil calcium content x moisture	0.47*	n.s.	0.67**	0.48*
Soil calcium content x E-moisture	0.47*	0.32*	0.68**	0.49*
E-reaction x E-moisture	n.s.	0.52**	n.s.	0.34*

E-nutrients ($r_s = 0.62$; $p < 0.001$), altitude ($r_s = 0.62$; $p < 0.001$), annual precipitation ($r_s = 0.61$; $p < 0.001$), and E-reaction ($r_s = 0.61$; $p < 0.001$)

(Table 4, Fig. 2). This change of snail species composition was also strongly associated with the main change of plant species composition

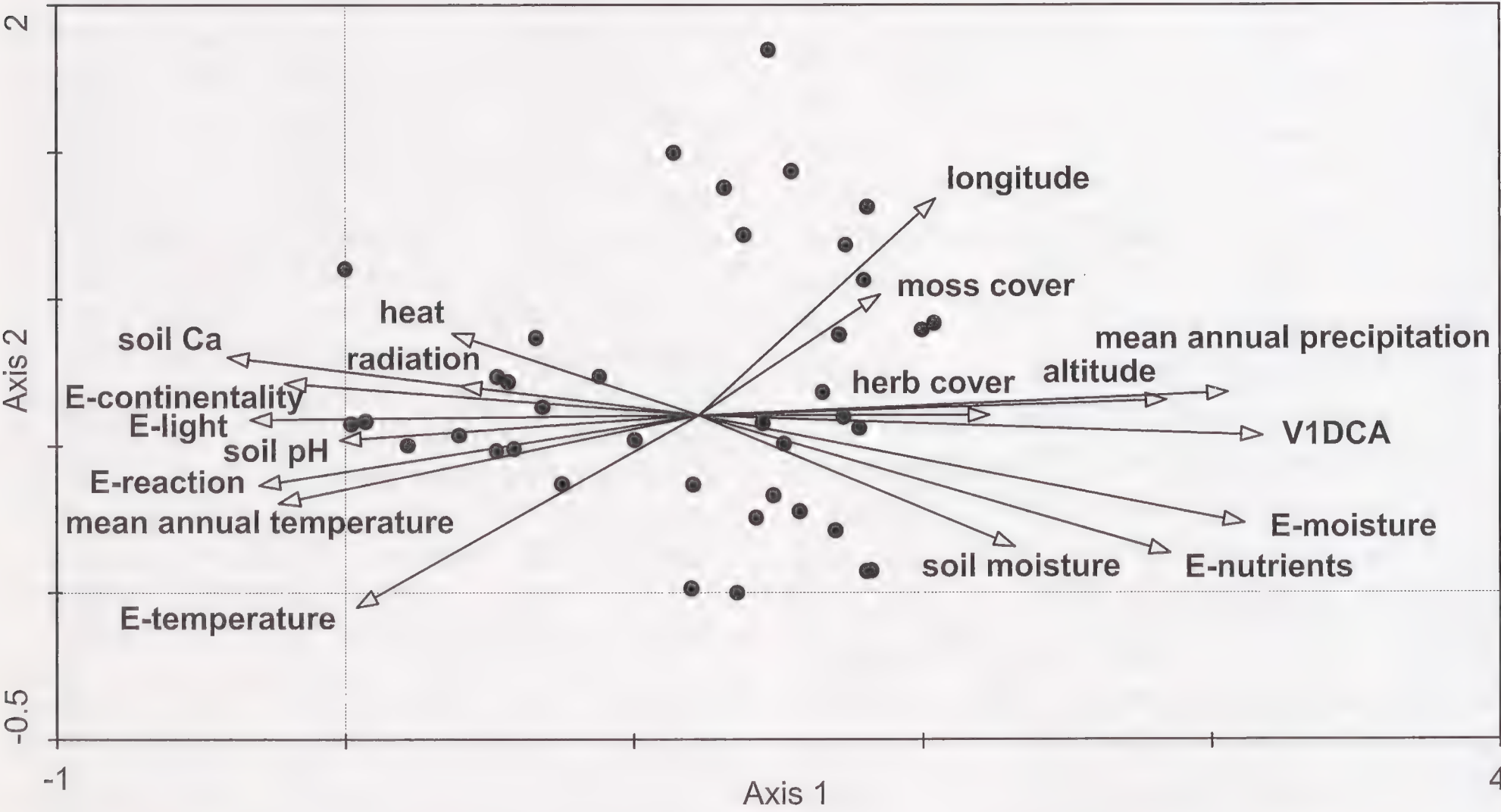


FIG. 2. Detrended correspondence analysis (DCA) of snail assemblages: ordination plot of sites on the first two DCA axes. The first two DCA axes explained 34.5% of total variation in snail data (first axis 24.7%). Only variables significantly related to species composition ($p < 0.05$) are shown.

TABLE 4. Correlations between the first two DCA axes and all explanatory variables. Significance is given as: n.s. - not significant; * - $p < 0.05$; ** - $p < 0.01$.

Explanatory variables	1st DCA	2nd DCA
Latitude	n.s.	n.s.
Longitude	0.47*	n.s.
Soil pH	-0.45*	n.s.
Soil calcium content	-0.51**	n.s.
Soil moisture	0.40*	n.s.
Altitude	0.62**	n.s.
Mean annual temperature	-0.55**	n.s.
Mean annual precipitation	0.60**	n.s.
Radiation	-0.38*	n.s.
Heat	-0.35*	n.s.
E-light	-0.52**	n.s.
E-temperature	-0.49*	-0.39*
E-continentality	-0.59**	n.s.
E-moisture	0.75**	n.s.
E-reaction	-0.61**	n.s.
E-nutrients	0.62**	n.s.
V1DCA	0.77**	n.s.
V2DCA	n.s.	n.s.
V3DCA	n.s.	n.s.
V4DCA	n.s.	n.s.
Moss cover	0.38*	0.33*
Herb cover	0.39*	n.s.

(V1DCA; $r_s = 0.77$; $p < 0.001$). Site scores on the second DCA axis of the snail data were only weakly correlated with E-temperature

and moss cover (Table 4). Calcium-poor but wet sites, lying often at high altitudes with high precipitation, were placed on the right side of the diagram (Fig. 2). At these sites we found only few common species in a low abundance, in particular *Perpolita hammonis*, *Vitrina pellucida*, *Vallonia pulchella* and *Cochlicopa lubrica* (Fig. 3). The calcium-rich but dry sites with open vegetation were on the left side of the diagram. These sites were inhabited by species that preferred sites with warm conditions. They were also species-poor, but they hosted such typical steppe species as *Granaria frumentum* and *Truncatellina cylindrica* (Fig. 3). These differences are linked with different responses of several species to the studied variables (Table 5). Abundance of *Granaria frumentum* and *Truncatellina cylindrica* were positively correlated with calcium content and negatively with moisture. In contrast, abundances of *Perpolita hammonis* and *Vitrina pellucida* were positively correlated with moisture and indifferent to calcium content.

Variation among snail assemblages was significantly associated with variables in all three analysed groups. Table 6 shows the nine variables which explained most variation in snail data and were used in partial CCA analyses. The resulting diagram (Fig. 4) shows that most of the variation in snail data was shared by all groups of variables (11%). Each group had its own contribution; however, the independent variation explained by EIVs was not significant. An exclusive and independent proportion of snail data variation was explained by the main change of plant species composition (V1DCA). A significant and exclusive contribution of soil calcium content within measured variables was also of high importance.

TABLE 5. Spearman correlations between species abundances and explanatory variables. Significance is given as: n.s. - not significant; * - $p < 0.05$; ** - $p < 0.01$. Abbreviation: GraFru, *Granaria frumentum*; PerHam, *Perpolita hammonis*; TruCyl, *Truncatellina cylindrica*; VitPel, *Vitrina pellucida*.

Explanatory variables	GraFru	TruCyl	PerHam	VitPel
Soil calcium content	0.61**	0.71**	n.s.	n.s.
Soil pH	0.55**	0.55**	n.s.	n.s.
E-reaction	0.58**	0.69**	n.s.	n.s.
Soil moisture	n.s.	-0.54**	n.s.	0.32*
E-moisture	-0.50*	-0.58**	0.51**	0.50*

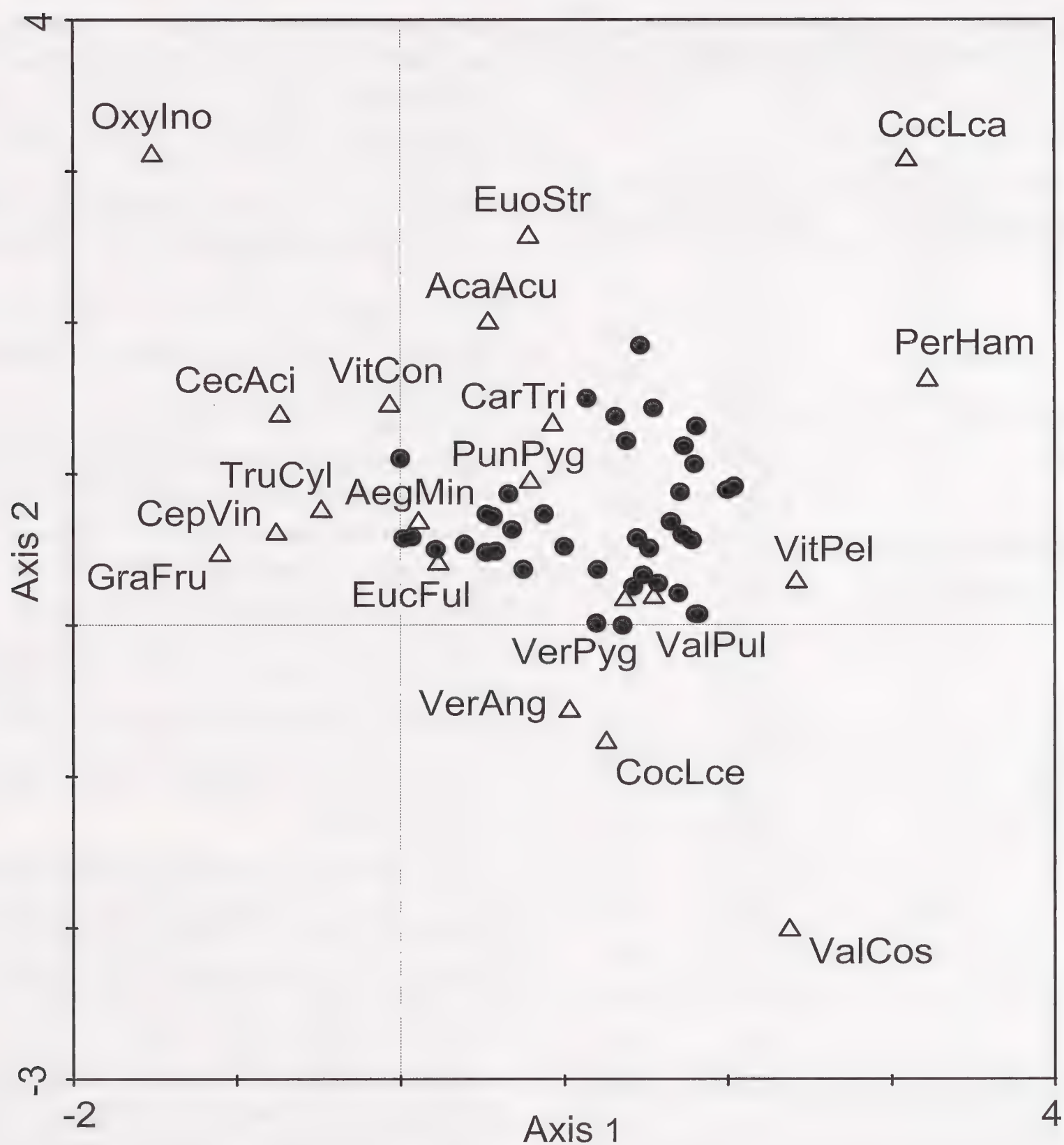


FIG. 3. Detrended correspondence analysis (DCA) of snail assemblages: ordination plot of species on the first two DCA axes. Abbreviation: AcaAcu, *Acanthinula aculeata*; AegMin, *Aegopinella minor*; CecAci, *Cecilioides acicula*; CepVin, *Cepaea vindobonensis*; CocLca, *Cochlicopa lubrica*; CocLce, *Cochlicopa lubricella*; EucFul, *Euconulus fulvus*; EuoStr, *Euomphalia strigella*; GraFru, *Granaria frumentum*; OxyIno, *Oxychilus inopinatus*; PerHam, *Perpolita hammonis*; PunPyg, *Punctum pygmaeum*; TruCyl, *Truncatellina cylindrica*; ValCos, *Vallonia costata*; ValPul, *Vallonia pulchella*; VerAng, *Vertigo angustior*; VerPyg, *Vertigo pygmaea*; VitCon, *Vitrea contracta*; VitDia, *Vitrea diaphana*; VitPel, *Vitrina pellucida*.

DISCUSSION

In this study, the relative importance of different variables in structuring the snail fauna was hard to determine because the relevant variables were highly intercorrelated (Table 1). The same conclusion has been reached in most studies on ecology of land snails (e.g., Labaune & Magnin, 2001). High positive correlations of variables reflecting similar conditions (calcium content, E-reaction, pH) were not surprising, but negative correlation of soil calcium content and moisture was a more interesting finding.

Most of the studied meadows were both calcium poor and wet and the calcium poorest and wettest sites lay in high-altitude areas with high precipitation. All those sites occurred on deep, originally forest soils and it is likely that they lost calcium carbonate due to cation leaching (Otýpková et al., 2011).

Snail species composition was strongly affected by variables expressing site moisture, calcium content, and altitude (Table 4). This finding is consistent with the results of many previous studies (Davies & Grimes, 1999; Horsák 2006; Martin & Sommer, 2004a, b)

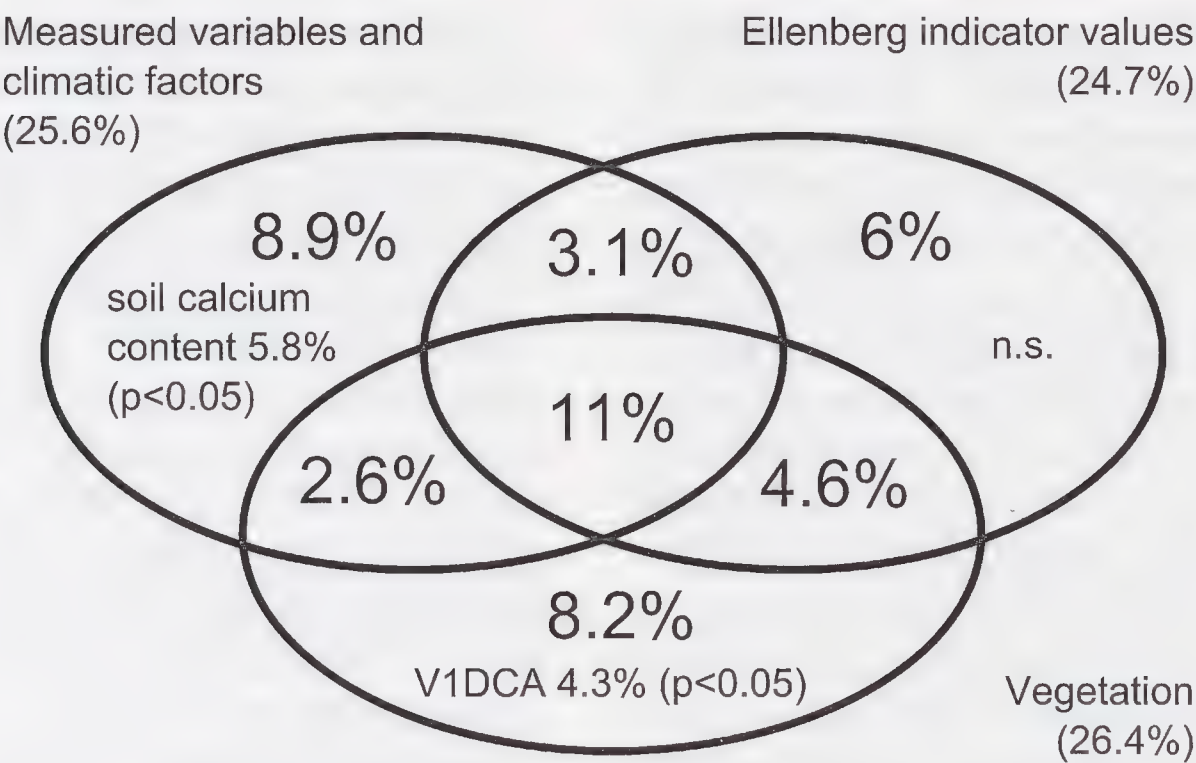


FIG. 4. Venn diagram of variation partitioning among three groups of variables (each consisting of three variables; see Table 6).

and confirms strong dependence of snails on site conditions. However, there are at least two reasons why it is impossible to disentangle the effects of three above mentioned factors. Firstly, these factors all reflect a single ecological gradient and all these factors are known to control snail species composition. The wetter sites were also calcium poor, and thus they supported only few, low abundant common species. Dry and calcium rich sites were also species poor; nevertheless, they hosted exclusively steppe species though they were too dry for the occurrence of other more common generalist species. We observed high predictive power of an interaction between soil calcium content and soil moisture for both snail abundances and species composition (Tables 3, 5), which was strengthened by various responses of individual species to these factors (Hermida et al., 1995; Ondina et al., 1998, 2004; Outeiro et al., 1993). In this study, low soil calcium content was the limiting factor for *Granaria frumentum* and *Truncatellina cylindrica*, low soil moisture was limiting factor for *Perpolita hammonis* and *Vitrina pellucida*.

Studies on spring fen communities, where the soil moisture is permanently high, suggested that *T. cylindrica* inhabits wet fens if calcium carbonate content is very high (Horsák, 2006; Horsák & Hájek, 2003). In contrast, *G. frumentum* occurred only in dry meadows with high soil calcium content. *Perpolita hammonis* and *V. pellucida* occurred along the whole mineral poor-rich gradient of the fens, but they had low abundance in the driest meadows or were totally absent there.

The main change in snail assemblage composition was explained not only by directly measured variables and climatic factors but also by their estimates on the basis of Ellenberg indicator values and by changes in plant species composition. It is obvious that snails and plants living at the same plot in the same time are affected by the same environmental conditions; thus, species composition of their assemblages is probably determined by similar environmental factors. This is in accordance with our results. We observed that the main change of snail species composition and plant species composition (both expressed as site

TABLE 6. Variables selected in forward selection (CCA, Monte Carlo test, $p < 0.01$) as the most important for explaining variation in snail species data and percentage of independent variation in snail data explained by each variable.

Measured variables and climatic factors		Ellenberg indicator values		Vegetation	
Mean annual precipitation	13.8%	E-moisture	13.4%	V1DCA	15.6%
Soil calcium content	6.8%	E-reaction	7.0%	V4DCA	5.6%
Soil moisture	5.0%	E-temperature	4.3%	V3DCA	4.8%

scores on the 1st DCA axis) were highly correlated ($r_s = 0.77$, $p < 0.001$). Consequently, we used the variance partitioning approach to analyse the extent to which it is possible to use vegetation as a predictor of land snail composition. We found that most variation in snail species data was shared by all three groups of variables: directly measured variables, changes in plant species composition, and Ellenberg indicator values (Fig. 4). However, pure effects of both vegetation and measured variables were significant. This indicates that vegetation can effectively describe those factors that were not recorded or probably that are difficult or even impossible to directly measure. These factors include among others historical development, site management and biogeographical patterns. Nevertheless, these hidden factors can influence both vegetation and snail assemblages in the same ways and thus shape their species composition. A high level of concordance in species composition of plants and snails was also found in the Western Carpathian spring fens, where vegetation was able to predict the composition of mollusc assemblages even better than measured water chemistry parameters (Horsák & Hájek, 2003). Historical development of these fens influenced distribution of relict plant and snail species, which are significantly related to old sites with Late Glacial continuity (Hájek et al., 2011). This is the main reason for a sharp geographical gradient within a rather small area, independently of any climatic or other environmental variation (Horsák et al., 2007a). Magnin et al. (1995) found similar patterns, related to the landscape dynamic effect on snail and plant assemblages.

In our study, the significant and exclusive contribution of soil calcium content shows that it is impossible to estimate completely the effect of calcium content based on vegetation and Ellenberg IVs. This raises an interesting question that plants and snails could uptake calcium from different soil layers. In most of the studied meadows, the surface layer is decalcified and the content of calcium increases with soil depth (Dvořáková, unpubl. data; Jongepierová, 2008). In contrast, Ellenberg soil reaction values were better correlated with the first DCA axis of snail species composition than soil pH and calcium content. The same pattern was observed by Horsák et al. (2007b), who identified Ellenberg soil reaction values as the best predictor of fen mollusc species composition. However, a pure effect of EIVs for soil reaction

in the present study was not significant, probably because of their similarity with the group of variables reflecting plant species composition gradients (expressed as three significant DCA axes; Fig. 4, Vegetation, 4.6%). Corresponding with Horsák et al. (2007b), Ellenberg indicator values for some environmental variables has even higher predictive power than directly measured values. A high level of intercorrelation between variables obtained from plant species composition and directly measured factors confirmed that data on vegetation can act as a proxy for the general environmental regime and its effect on snail faunas.

However, it is not surprising that in general it is difficult to disentangle the influence of vegetation structure (i.e., mean height, proportion of bare ground, proportion of vascular plant and bryophyte cover) and species composition because vegetation structure strongly depends on particular plant species, and plant species occurrences are controlled by vegetation structure due to the competitive exclusion. In our study, we used the cover of herbs and mosses as variables describing vegetation structure, but we suggest that better information about vegetation structure is contained mostly in species composition. Several studies showed that also occurrence and abundance of snail species dependent on vegetation structure mainly because of food source, microclimate and presence of available shelters (Boycott, 1934; Cameron et al., 1980; Labaune & Magnin, 2001). However, these studies pointed out that the possible relationships between snail and plant assemblages are more dependent on microclimate conditions controlled by vegetation structure than on plant species composition itself. On the contrary, our results support different hypothesis that even vegetation composition can be very useful predictor of variation in snail assemblages.

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AGE AND GROWTH OF *OLIVANCILLARIA DESHAYESIANA* (GASTROPODA: OLIVIDAE) IN THE SOUTHWESTERN ATLANTIC OCEAN

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ABSTRACT

The population structure, particularly growth, age, mortality and somatic production of the olivid snail *Olivancillaria deshayesiana* were investigated. Annual formation of internal shell growth marks was confirmed by the record of stable oxygen isotopes in the shell, which reflects seasonal patterns of water temperature. A von Bertalanffy growth model fitted to 81 size-at-age data pairs, indicating that *O. deshayesiana* may attain 31 mm SL in about 10 years. The estimated total mortality Z and natural mortality M were 0.651 y^{-1} and 0.361 y^{-1} , respectively. Fishing mortality F was 0.290 y^{-1} , and the exploitation rate E was 0.445, indicating that this population was not overexploited at the time of the study. However, this situation may well change in the future, since the important prawn and shrimp fisheries (in intensity and scale) in the Mar del Plata area ($38^{\circ}20'S$, $57^{\circ}37'W$) may indirectly affect the exploitation status of the studied population.

Key words: marine gastropod, age, growth, mortality, production.

INTRODUCTION

The determination of the individual age within a population is important for a comprehensive understanding of population dynamics and is required to establish growth and mortality patterns. Several methods have been used to study growth in gastropods (e.g., Shigemiyama & Kato, 2001; Richardson et al., 2005a, b; Barroso et al., 2005), including the family Olividae (Stohler, 1969; Tursch et al., 1995; Rocha-Barreira, 2003; Caetano et al., 2003). In recent years, the technique by Epstein et al. (1953) based on the analysis of the isotope composition of calcium carbonate shells has been increasingly used by many authors (Giménez et al., 2004; Cledón et al., 2005; Bigatti et al., 2007; Arrighetti et al., 2010).

The family Olividae is composed of marine gastropods of moderate size, rarely exceeding 10 cm in total shell length. They inhabit sandy coastal shores in tropical and temperate regions of the world (Smith, 1998). There are eight *Olivancillaria* species in the southwestern Atlantic Ocean (Teso & Pastorino, 2011), some of which are the target of subsistence

fisheries (Scelzo et al., 2002; Narvarte, 2006). This family, together with some volutids (i.e., *Adelomelon*, *Zidona*) and nassarids (i.e., *Buccinanops*) represent the most important endemic gastropods of sandy bottoms in the Argentine malacological province.

Olivancillaria deshayesiana is a small gastropod (up to 35 mm) distributed from Rio de Janeiro ($22^{\circ}59'S$, $43^{\circ}11'W$), Brazil, to Necochea ($38^{\circ}33'S$, $58^{\circ}44'W$), Argentina, at 0–60 m depth (Teso & Penchaszadeh, 2009; Teso & Pastorino, 2011). Individuals are semi-buried in the substratum, and their shell is partially covered by the mantle. The surface of the shell exhibits a distinct pattern of growth bands that have been used for ageing purposes, as in *O. auricularia* (Rocha-Barreira, 2003). In addition, Caetano et al. (2003) estimated growth and mortality of *O. vesica* from length-frequency data with computer-based methods.

Little is known about the reproduction and ecology of *O. deshayesiana*. Borzone (1995) described its embryonic development and egg capsules, and Teso & Penchaszadeh (2009) reported the imposex phenomenon in a population from the Mar del Plata area. This species

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is taken as bycatch in the fisheries for prawn *Artemesia longinaris* and shrimp *Pleoticus muelleri*, rather than being the direct target of artisanal fishermen (Scelzo et al., 2002). The objective of this study was to determine growth, mortality and the individual production of *O. deshayesiana* in the Mar del Plata area and to evaluate its significance for the local fishery.

MATERIALS AND METHODS

Specimens of *Olivancillaria deshayesiana* were collected monthly by bottom trawl (3 m mouth opening, 15 mm of mesh size) off Mar del Plata City (38°20'S, 57°37'W) between 5 and 12 m depth between October 2005 and September 2007. In this area, water temperature ranges between 8.4 and 20.8°C and salinity is rather constant at 33.8‰ (http://www.iafe.uba.ar/tele/Antares_argentina/; Guerrero & Piola, 1997). Animals were taken to the laboratory, where the shell was separated from the soft body. Shell length (SL) was measured with a vernier caliper to the nearest 1 mm and spiral growth trajectory length (SG) with a thread to the nearest 1 mm (Fig. 1). Shell-free wet mass (SFWM) was weighed to the nearest 0.1 g. The relationship between SL and SFWM was calculated.

Age and Growth

Only the pattern of internal growth marks was considered for the analysis due to the presence of columellar material forming a callus on the spire. The shells of ten individuals were cut along the whorls following the spiral growth trajectory from the apex to the posterior end of the aperture (Fig. 1), as described by Bigatti et al. (2007) and Arrighetti et al. (2010), among others. The cut surface was ground with sandpaper, using grits of P400, P1200, P2400 and P4000 grade. The polished shell cuts were examined by stereomicroscope. Visible internal growth marks were identified and the corresponding growth trajectory length SG_{*i*} from the apex to each mark *i* was measured as described by Cledón et al. (2005) and Gurney et al. (2005), among others. A stable oxygen isotope ratio ($\delta^{18}\text{O}$) was used to confirm the annual periodicity in the formation of growth marks. $\delta^{18}\text{O}$ in biogenic calcium carbonate structures is inversely proportional to temperature during carbonate deposition, as demonstrated empirically by Epstein & Lowenstam (1953) and Epstein et al. (1953).

Two representative specimens of 31 mm and 29 mm SL were used for stable isotope analysis. The carbonate samples ($\pm 50 \mu\text{g}$ each) were collected from the central shell layer along the SG by means of a dental drill (bit size 0.5 mm)

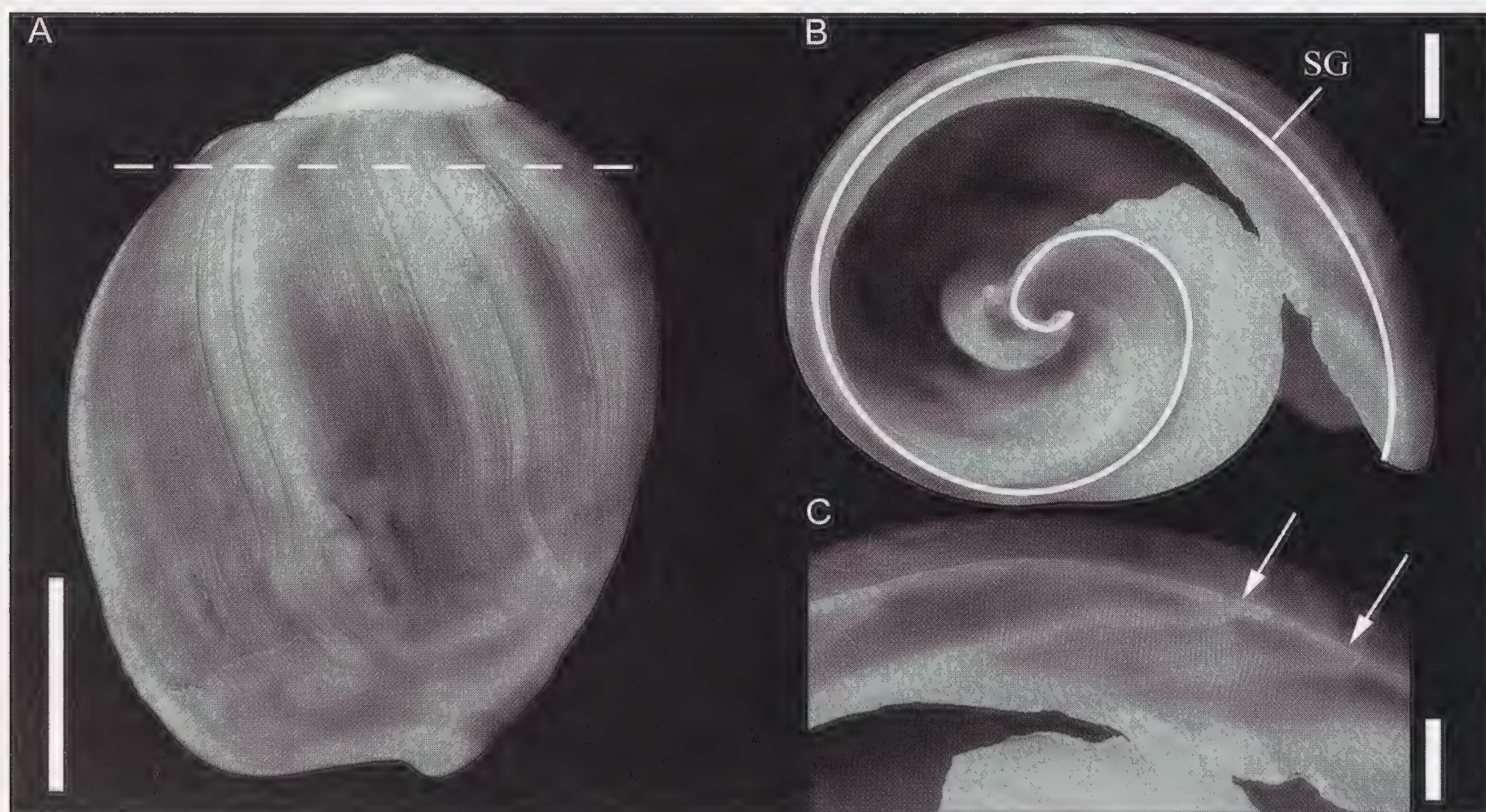


FIG. 1. *Olivancillaria deshayesiana*. FIG. 1A, B: Showing the spiral growth trajectory (SG), along which the samples for oxygen and carbon isotopes were taken, dash line in A indicates location of section in B; FIG. 1C: Detail of the internal growth marks (arrows). Scale bar = 1 cm.

at a sample-to-sample distance of about 1.5 mm. Stable oxygen isotope composition was determined in the stable isotope laboratory of the Alfred Wegener Institute with a Finnigan MAT251 mass spectrometer coupled to an automatic carbonate preparation device. The precision of measurements was better than $\pm 0.08\text{‰}$ for $\delta^{18}\text{O}$ based on repeated analysis of a laboratory-working standard over a one-year period. Data are related to the Pee Dee belemnite (PDB) standard through repeated analyses of National Bureau of Standard (NBS) isotopic reference material 19 by:

$$\delta = [(R_{\text{sample}} / R_{\text{standard}}) - 1] * 1000$$

where R_{sample} and R_{standard} are the isotopic relationship of the sample and of the standard, respectively. $\delta^{18}\text{O}$ ($d^{18}\text{O}_c$) values were converted into water temperature by the paleotemperature equation of Epstein et al. (1953), as modified by Craig (1965) and Anderson & Arthur (1983):

$$T(^{\circ}\text{C}) = 16.00 - 4.140 * (d^{18}\text{O}_c - d^{18}\text{O}_w) + 0.130 * (d^{18}\text{O}_c - d^{18}\text{O}_w)^2$$

Sea water $\delta^{18}\text{O}$ ($d^{18}\text{O}_w$) was estimated at -0.8‰ from the gridded data (version 1.1) of LeGrande & Schmidt (2006); see also the corresponding NASA website (<http://data.giss.nasa.gov/o18data>). Visual shell marks coinciding with $\delta^{18}\text{O}$ peaks were interpreted as winter growth marks, and the distance between two subsequent winter growth marks as the annual shell increment. Thus, each winter growth mark i and its corresponding shell growth trajectory length SG_i were interpreted as one size-at-age data pair. SG_i was translated into shell length SL_i by means of the linear relationship between the two parameters. The iterative non-linear Newton algorithm was used to fit a growth model to the size-at-age data. The specialized von Bertalanffy growth model was found to fit the data best:

$$SL_t = SL_{\infty} * (1 - e^{-K*(t-t_0)})$$

where SL_{∞} is asymptotic shell length, K is the body growth coefficient, t is age and t_0 is a theoretical age at which length equals zero. Overall growth performance was estimated by the index:

$$P = \log(K * SL_{\infty}^3)$$

as described by Moreau et al. (1986) & Pauly et al. (1994).

Production

Individual somatic production was calculated by the mass-specific growth rate method ac-

cording to Brey (2001) from the size frequency distribution, the size-body function and the growth model parameters:

$$P_j = BM_j * G_j$$

where BM_j is the mean individual body mass at size j and G_j is the annual mass specific growth rate at size j as calculated by:

$$G_j = b * K * (SL_{\infty} / SL_j - 1)$$

Smaller size classes are likely to be under-sampled due to the selectivity of the commercial bottom trawl, and thus population production (P) was not calculated.

Mortality, Yield per Recruit and Exploitation Rate

It was presumed that mortality in *O. deshayesiana* is adequately described by the single negative exponential mortality model and the corresponding size-converted catch curve (Pauly, 1984a, b) was used to estimate total mortality rate Z (y^{-1}),

$$N_j / \Delta t_j = N_0 * e^{-Z t_j}$$

where N_j is number in size class j , Δt_j is time required to grow through size class j , and t_j is age at midsize of size class j . Natural mortality rate M was estimated by means of an empirical model that relates production-to-biomass ratio (P/B) ratio to maximum age t_{max} (y), maximum body mass BM_{max} (kJ) and mean water temperature T (Kelvin) in unexploited benthic populations (Brey, 1999, 2001). If growth can be described by the von Bertalanffy model and mortality by the single negative exponential model, mortality rate M and P/B ratio are equivalent (Allen, 1971), hence:

$$\log(M) = 1.672 + 0.993 * \log(1/t_{\text{max}}) - 0.035 * \log(BM_{\text{max}}) - 300.447/T$$

$$N = 901, r^2 = 0.880$$

Maximum age and body mass were inferred from the largest animal through the growth model and the size-mass relationship (applying the conversion factor 3.818 kJ/gWM, average for Gastropoda, Brey, 2001).

Fishing mortality F and exploitation rate E were computed by:

$$F = Z - M, \text{ and } E = F/Z$$

A selection curve giving the probability of capture PC_j per size class j was computed by a procedure outlined by Pauly (1984a, b). Yield per recruitment was computed by the Beverton & Holt (1964) model, from which a predicted value for optimum exploitation rate was also obtained.

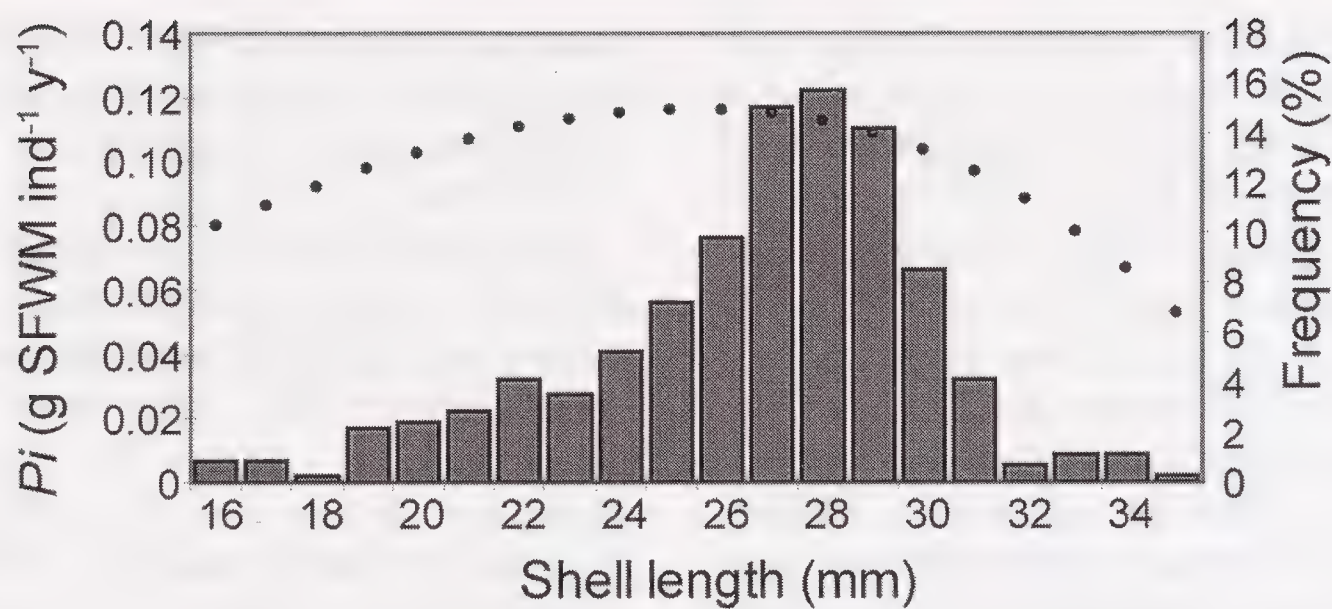


FIG. 2. Size-frequency distribution of *Olivancillaria deshayesiana* (N = 420) from Mar del Plata area in 2005–2007. Superimposed dotted line indicates individual somatic production P_i (g shell-free wet mass year⁻¹) per size class.

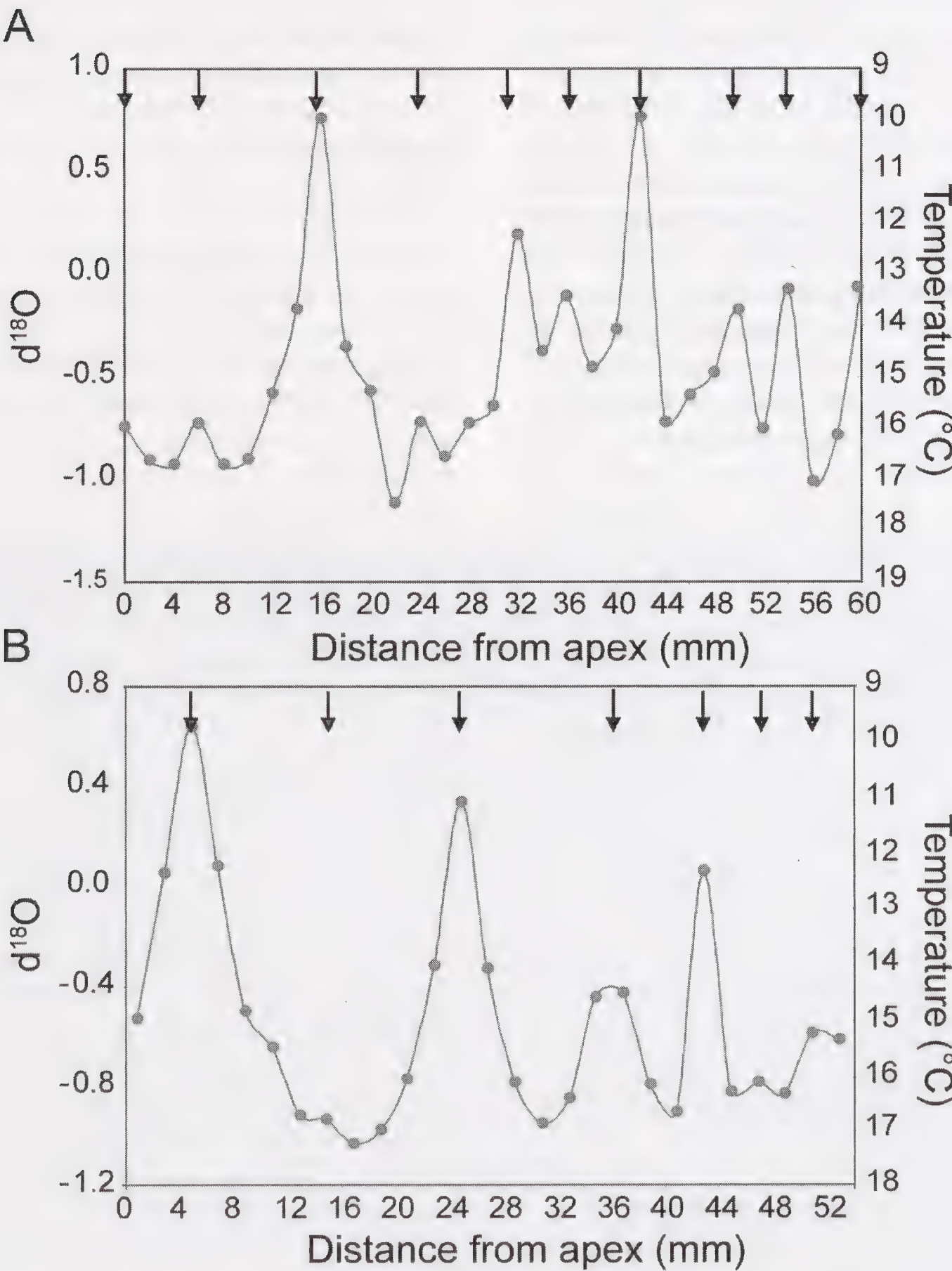


FIG. 3. $\delta^{18}O$ profile along a transect from apex to shell edge from two individuals of *Olivancillaria deshayesiana*. FIG. 3A: 31 mm SL, 10 y; FIG. 3B: 29 mm SL, 7 y. Arrows indicate position of internal growth marks. Water temperature (right-hand scale) computed from carbonate ^{18}O by the paleotemperature equation of Epstein et al. (1953); see text for details.

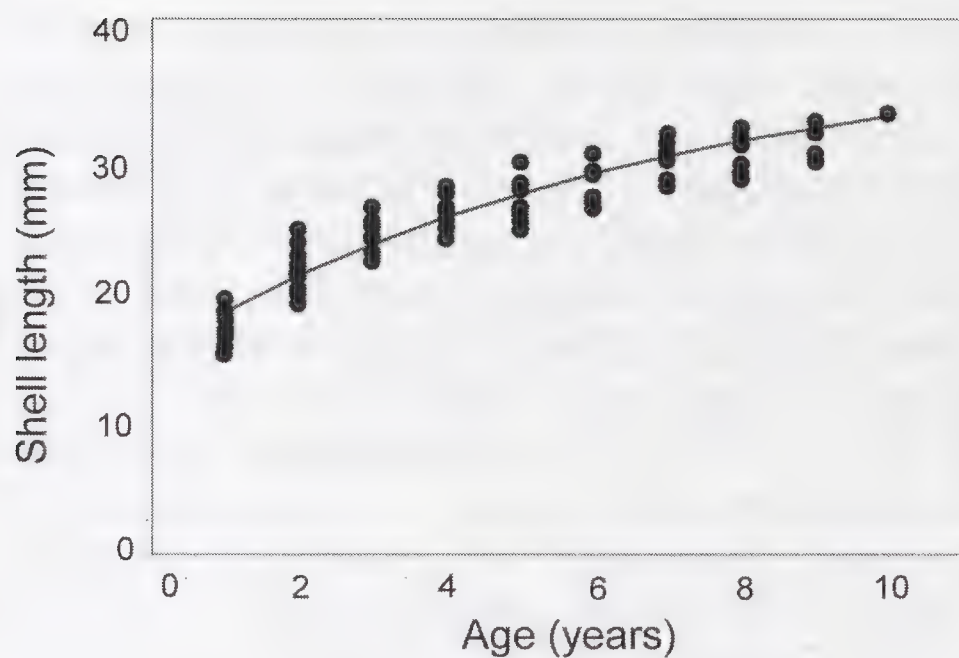


FIG. 4. Von Bertalanffy growth curve for *Olivancillaria deshayesiana* $SL_t = 38 * (1 - e^{-0.147*(t-3.392)})$; $r^2 = 0.913$; $N = 81$ size-at-age data pairs obtained from the 10 specimens analyzed.

RESULTS

A total of 470 snails were collected, which ranged from 16 to 35 mm in total shell length (Fig. 2). Shell-free wet body mass ranged from 0.4 to 10.8 g and was related to *SL* by:

$\log(SFWM) = -4.76 + 3.60 * \log(SL)$; (g – mm; $r^2 = 0.77$; $N = 470$)

SL was linearly related to *SG*, the distance from the apex along the growth trajectory to the aperture of the shell, by:

$SL = -42.87 + 3.54 * SG$; (mm – mm; $r^2 = 0.93$; $N = 30$)

Age and Growth

The $\delta^{18}O$ profiles showed distinctly oscillating pattern in both shells (Fig. 3). The average

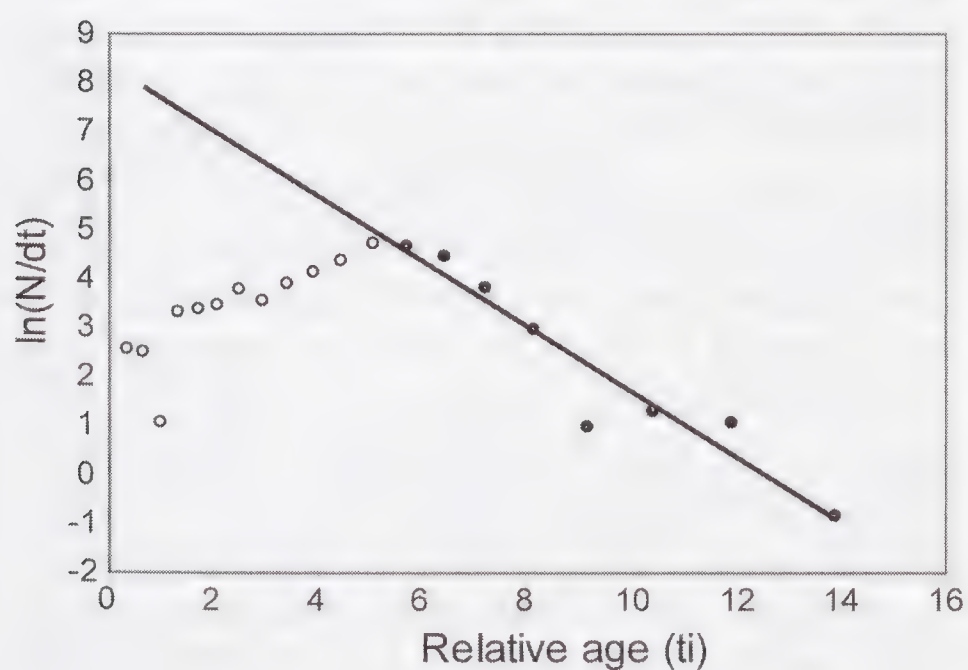


FIG. 5. Size-converted catch curve based on the size-frequency distribution (Fig. 2) and growth function (Fig. 4) of *Olivancillaria deshayesiana*. Dots: data included in regression; circles: data excluded from regression. $\ln(N/dt) = -0.651 ti + 8.193$; $r^2 = 0.93$; 95% confidence range of slope = ± 0.164 .

range of -1.1‰ to $+0.8\text{‰}$ $\delta^{18}O$ corresponded to a temperature range of about 9.8°C to 17.4°C . Almost all $\delta^{18}O$ maxima (corresponding to temperature minima) coincided with a shell growth mark, thereby indicating that one growth mark is formed each winter.

The von Bertalanffy growth model:

$SL_t = 38 * (1 - e^{-0.147*(t-3.392)})$; $N = 81$, $r^2 = 0.913$

fitted best the 81 size-at-age data pairs obtained from the 10 specimens analyzed (Fig. 4). Overall growth performance of *O. deshayesiana* was $P = 3.91$.

Production

Individual somatic production P_j increased with size j up to a maximum of 0.116 g SFWM year $^{-1}$ at 25 mm *SL* and decreased towards larger sizes (Fig. 2).

Mortality, Yield per Recruit and Exploitation Rate

Total mortality rate Z of *O. deshayesiana* amounted to 0.651 y $^{-1}$ (95% confidence range ± 0.164 , Fig. 5). Natural mortality rate M was estimated at 0.361 y $^{-1}$, with $t_{max} = 20.7$ y (at 35 mm *SL*), $BM_{max} = 41.23$ kJ (10.8g SFWM) and $T = 286$ K. Accordingly, fishing mortality F amounted to 0.290 y $^{-1}$ and exploitation rate E to 0.445 . Figure 6A shows the selection curve for the currently applied sampling technique. Specimens less than 17 mm were not caught at all; probability of capture attained 50% at 22.8 mm *SL* and 100% ≥ 27 mm *SL*. The optimum exploitation rate was estimated at 0.770 (Fig. 6B).

DISCUSSION

Stable Oxygen Isotope Ratios and Environmental Temperature

In the shell of *O. deshayesiana*, stable oxygen isotope ratios reflect seasonal patterns of water temperature; each maximum $\delta^{18}O$ value corresponds to a winter minimum temperature. Thus, the distinct shell mark at each $\delta^{18}O$ maximum indicates a slowing shell growth during winter, as observed for other gastropods from the Mar del Plata region (Cledón et al., 2005; Giménez et al., 2004). The average annual temperature inferred from the $\delta^{18}O$ profile in the shell ranged between 9.8°C and 17.4°C , in agreement with the reported seasonal instrumental temperatures of 8.4 – 20.8°C in the same area (http://www.iafe.uba.ar/tele/Antares_argentina/).

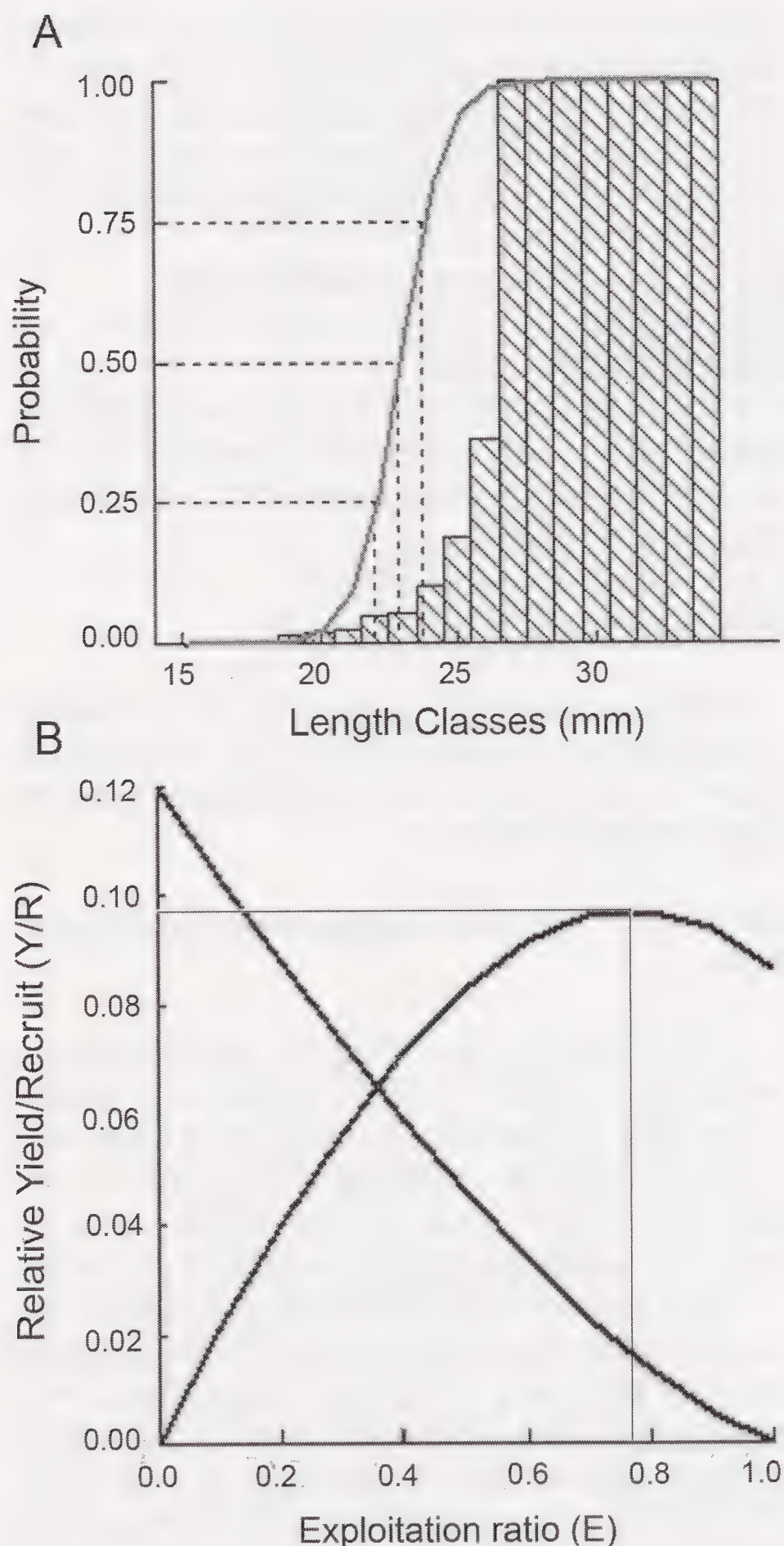


FIG. 6. *Olivancillaria deshayesiana*. FIG. 6A: Plot of probability of capture versus length in *Olivancillaria deshayesiana*; FIG. 6B: Yield per recruitment in *Olivancillaria deshayesiana* based on the Beverton and Holt (1964) model (calculations by means of the FISAT software reference).

Age and Growth

From the isotopic data and the internal growth marks, it is inferred that *O. deshayesiana* may reach 31 mm SL in about 10 years. This maximum age is similar to those reported for *Olivella biplicata* (8–12 years) from California, USA (Stohler, 1969) and *Oliva oliva* (10 years) from Papua, New Guinea (Tursch et al., 1995), while it is distinctly above those estimated for *O. auricularia* (6 years) from Rio Grande, Brazil (Rocha-Barreira, 2003) and *O. vesica* (4–5 years) from Rio de Janeiro, Brazil (Caetano

et al., 2003). In general, cold-water marine invertebrates found at higher latitudes grow more slowly and live longer than closely related species occurring in warmer waters (Dehnel, 1955; Ray, 1960; Fonseca et al., 2000). This may account for the shorter life span of *O. vesica* compared with species of the same genus living at higher latitudes.

The specialized von Bertalanffy model describes the shell growth of *O. deshayesiana* properly, because it is a gastropod in which shell growth has no inflexion point (e.g., Frank, 1965; Caetano et al., 2003; Ilano et al., 2004; Richardson et al., 2005a; Chatzinikolaou & Richardson, 2008; Eversole et al., 2008; Arrighetti et al., 2011). This is in contrast to other gastropods with a sigmoid pattern of shell growth (e.g., Giménez et al., 2004; Cledón et al., 2005; Bigatti et al., 2007). Therefore, all standard fishery analysis based on the von Bertalanffy model, such as virtual population analysis, applies to *O. deshayesiana*.

The overall growth performance of *O. deshayesiana* ($P = 3.91$) is below the range found for other large commercially important gastropod species from temperate regions (reviewed in Arrighetti et al., 2011).

Production, Mortality and Exploitation Rate

The size-frequency distribution was based on data from catches with nets of 15 mm mesh size, and therefore sampling efficiency was lower than 50% for individuals smaller than 23 mm SL (Fig. 6A). This is clearly seen in the size-converted catch curve, where individuals younger than 5 years (27 mm SL) are under-represented.

Therefore, estimates of production and productivity based on this distribution would distinctly underestimate true population values. The estimated fishing mortality of 0.290 y^{-1} and the current exploitation rate of 0.445 indicate that this population was not overexploited at the time of the study. However, this situation may well change in the future, since the important prawn and shrimp fisheries (in intensity and scale) in the Mar del Plata area (Scelzo et al., 2002) may indirectly affect the exploitation status of the studied population. Furthermore, *O. deshayesiana* is a food source for commercial fishes such as *Urophycis brasiliensis* (Brazilian codling), *Sympterigia bonapartei* (ray), *Callorynchus callorynchus* (elephant fish) and *Micropogonias furnieri* (white croaker) (Penchaszadeh et al., 2006). It is possible that the depletion of the population may cause a shift to a new stable trophic situation.

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RESEARCH NOTE

TELLIMYA TEHUELCHA, NEW SPECIES: FIRST RECORD OF
TELLIMYA BROWN, 1827, IN SOUTH AMERICA (BIVALVIA: LASAEIDAE),
WITH NOTES ON LIFE HISTORY AND REPRODUCTION

Diego G. Zelaya^{1, 2*} & Cristián Ituarte³

INTRODUCTION

Galeommatoids are small bivalves usually living as epibionts on other invertebrates. Among them, a number of genera, such as *Waldo* Nicol, 1966, *Scioberetia* F. Bernard, 1895, *Montacuta* Turton, 1822, *Tellimya* Brown, 1827, *Brachiomya* Jespersen, Lützen & Nielsen, 2004, and *Montacutella* Jespersen, Lützen & Nielsen, 2004, are associated with sea urchins. Only *Scioberetia* and *Waldo* were reported from the southern tip of South America (F. Bernard, 1895a–c; Zelaya & Ituarte, 2002).

Tellimya was proposed to include *Mya suborbicularis* Montagu, 1803, *Ligula substriata* Montagu, 1808, *Mya ferruginosa* Montagu, 1808, *Tellimya lactea* Brown, 1827, *T. tenuis* Brown, 1827, *T. elliptica* Brown, 1827 (based on *Mya ferruginosa* Montagu, 1808, and an objective synonym thereof), *T. glabrum* Brown, 1827, and *T. ovata* Brown, 1827. Subsequently, Gray (1847) designated *Mya ferruginosa* as its type species. Pérès (1937), Pophan (1940), Deroux (1961) and Oldfield (1961) provided information on the gross anatomy and functional morphology of the type species, and Kamenev (2008) clarified the details of the hinge morphology of the genus. *Tellimya* has sometimes been regarded as a subgenus of *Montacuta* (e.g., Pelseneer, 1925; Ponder, 1968), but currently regarded as a full genus (e.g., Chavan, 1969; Aartsen, 1997; Marshall, 2002; Kamenev, 2008).

In the present paper, a new species of *Tellimya* from the Magellan Region, which constitutes the first record of the genus in South America, is described.

MATERIALS AND METHODS

The specimens described here were obtained at irregular intervals between March 2009 and May 2010 from the intertidal zone at Puerto

Deseado, Santa Cruz Province, Argentina (Fig. 1) attached to irregular echinoids of the genus *Abatus*. Echinoids were hand collected during maximum low tides and transported to the laboratory for inspection under stereoscopic microscope. The number of bivalves and their position on the echinoid test were recorded. The maximum diameter of echinoids was measured. Length (L: maximum anteroposterior distance), height (H: maximum dorsoventral distance perpendicular to L) and width (W: maximum distance across valves) of bivalves were measured. Mean values and standard deviation (SD) for the H/L and W/L ratios were calculated. Specimens for anatomy were fixed and decalcified in a 10% formalin solution with 5% acetic acid, and dissected under a stereoscopic microscope. Specimens for histology were fixed in Bouin's solution, dehydrated, embedded in Historesin (Leica®) and sectioned (3.5 µm thick). Sections were stained with haematoxylin and eosin. Shell morphology was studied and illustrated with scanning electron microscopy.

Voucher specimens were deposited in the collections of Invertebrate Zoology at Museo Argentino de Ciencias Naturales (MACN) and Museo de La Plata (MLP).

SYSTEMATICS

Tellimya tehuelcha, n. sp.

Figs. 2–33

Type Locality: 47°45'S, 65°52'W, Puerto Deseado, Santa Cruz Province, Argentina.

Type Material: Holotype (MLP 13396) and 20 paratypes (10 paratypes, MACN-In 39321; 10 paratypes MLP 13397).

Additional Material: Histological sections of seven specimens (MACN-In 39320).

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FIG. 1. Locality map: Latitude (°S), longitude (°W).

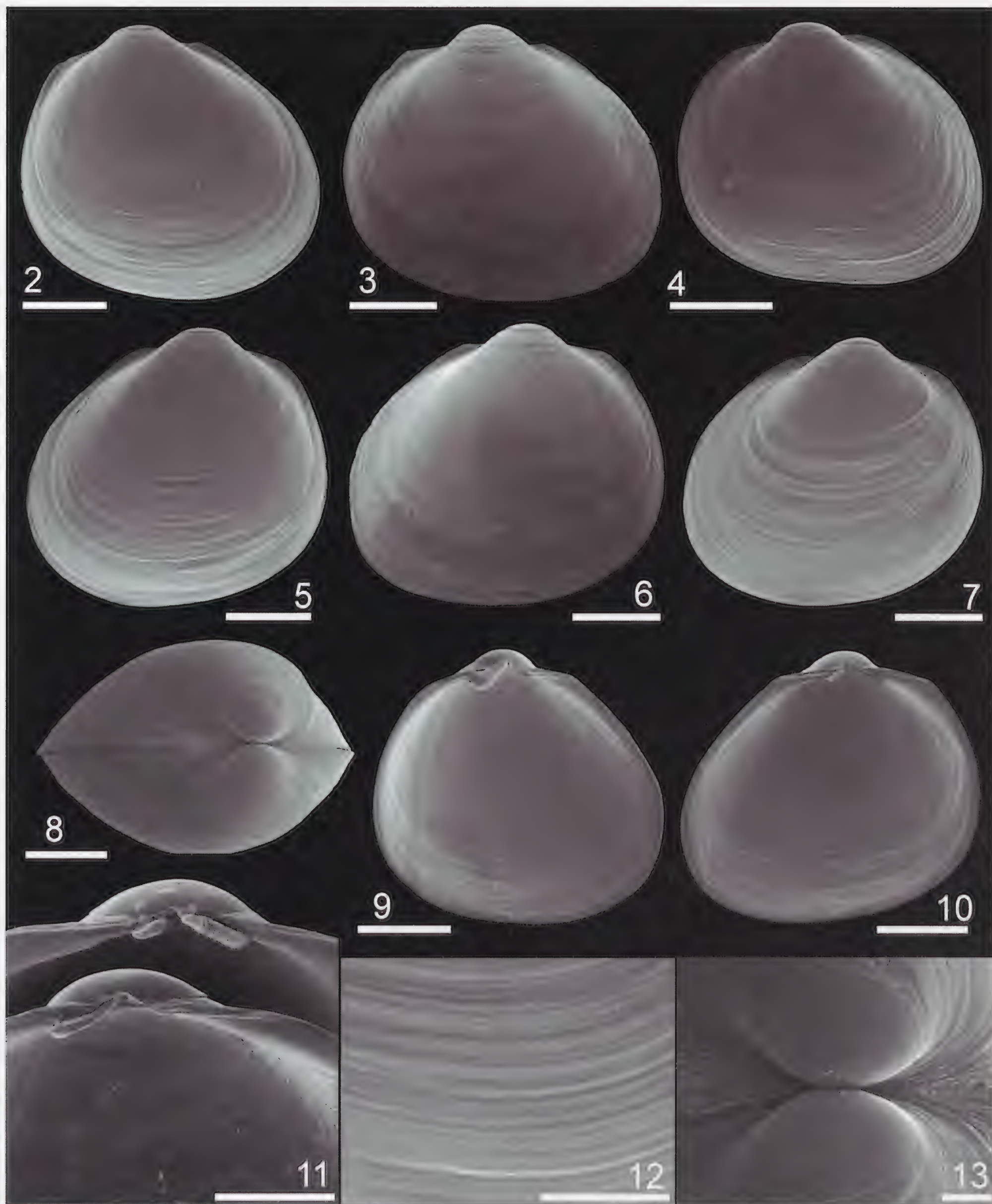
Distribution: Only known from the type locality (Fig. 1).

Etymology: The species name refers to the Tehuelches, one of the aboriginal people inhabiting vast regions of southeastern Patagonia.

Diagnosis: Shell high, trapezoidal, posteriorly truncate, inflated, sculptured only with well-marked growth lines. Anterior right cardinal

tooth short. Protoconch large (about 700 μm diameter). A single demibranch, with up to 50 filaments with extended and anastomosed adfrontal portions, present. Mantle border expanded anteriorly to delimit an inhalant area; inner fold with small papillae exteriorly visible in live specimens.

Description: Shell solid, small (maximum observed $L = 5.9$ mm), markedly trapezoidal in larger specimens ($H / L = 0.86 \pm 0.03$; $n =$



FIGS. 2–13. *Tellimya tehuelcha*, n. sp., shell morphology. FIGS. 2, 5: Holotype (MLP 13396); FIGS. 3, 4, 6–13: Paratypes (MLP 13397); FIGS. 2–8: Outer view. FIGS. 2–4: Right valve. FIGS. 5–7: Left valve. FIG. 8: Dorsal view; FIGS. 9–11: Inner views. FIG. 9: Left valve. FIG. 10: Right valve. FIG. 11: Details of right (upper) and left (lower) hinge plates; FIG. 12: Detail of shell sculpture; FIG. 13: Protoconch. Scale bars: Figs. 2–10 = 1 mm; Figs. 11, 12 = 500 μ m; Fig. 13 = 200 μ m.

10) (Figs. 2, 3, 5, 6, 9, 10), obliquely ovate in smaller specimens (Figs. 4, 7); inflated ($W/L = 0.61 \pm 0.03$; $n = 10$) (Fig. 8), equivalve, inequilateral. Anterior end rounded, projecting downward; posterior end truncate (Figs. 2–7). Dorsal margin short, nearly straight, horizontal. Posterior part of dorsal margin slightly sloping, forming a marked angle with posterior shell margin. Dorsal part of anterior margin long, suberect, sloping markedly; ventral part of anterior margin rounded, continuous with ventral margin. Ventral margin widely curved. Posterior margin widely arcuate or nearly straight, almost vertical to slightly oblique (Figs. 2–7). Beaks prominent, inflated, relatively low, posteriorly displaced, orthogyrate. Protoconch ovate, smooth, about 700 μm in diameter, well discernible from teleoconch (Fig. 13).

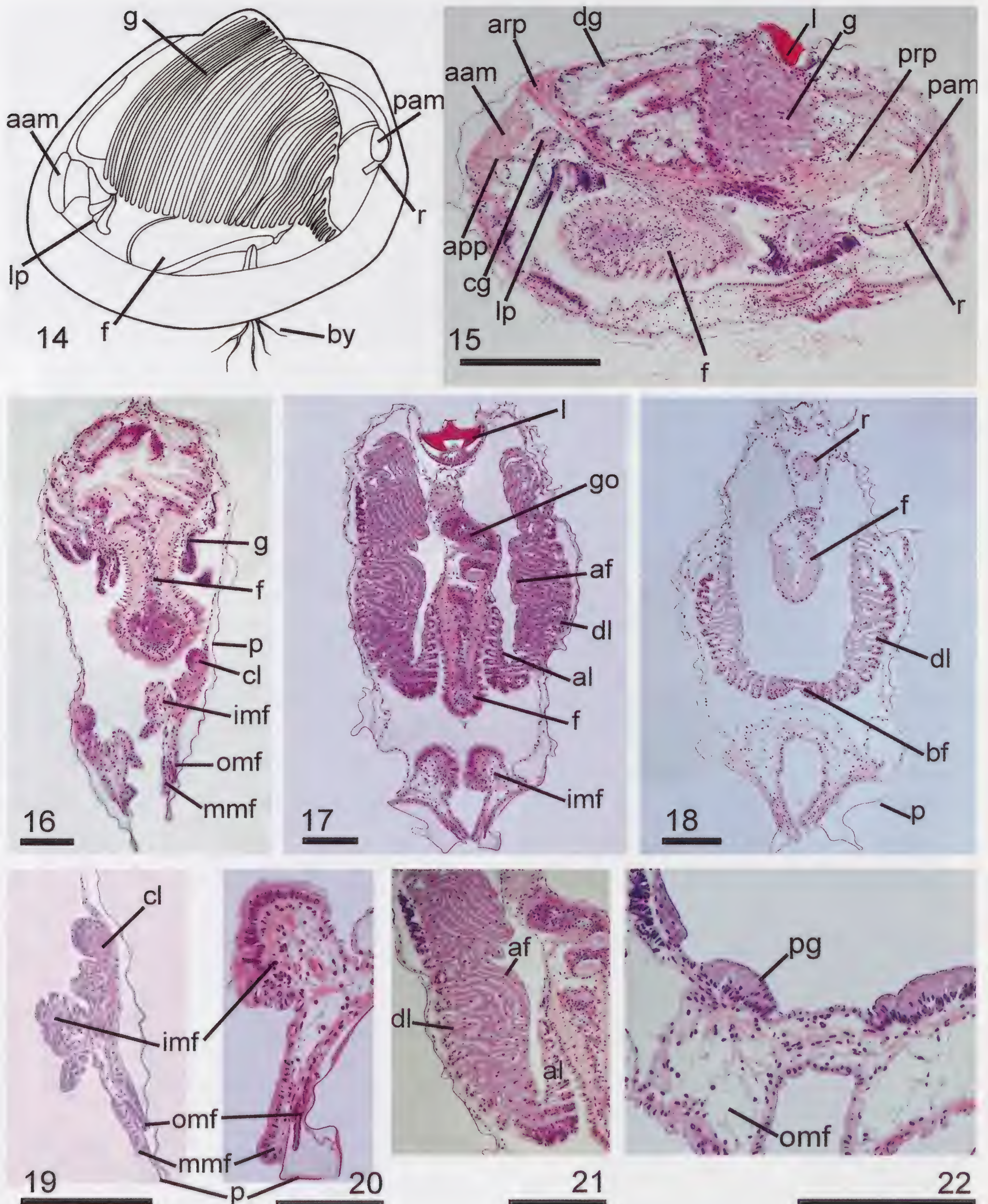
Shell surface white, brilliant, sculptured with well-marked, irregularly distributed growth lines (Fig. 12); radial striation absent; periostracum thick, translucent. Hinge plate interrupted behind beaks by a deep triangular pit (Fig. 11). Left valve with only one, anterior cardinal tooth that is slender, elongate, nearly horizontal, straight; right valve: only one anterior cardinal tooth that is short, oblique, moderately solid, cuneiform, slightly bent upward, with sharp cusp (Figs. 9–11). Resilifer short, wide, deeply notched in the cardinal platform, located just in front of posterior part of hinge plate. Resilium large, strong, posterior to beak, butterfly-shaped in transverse section, non-calcified (Figs. 9, 17). Adductor muscle scars and pallial line hardly visible.

Anatomy: Animal white. Mantle margin relatively wide, not reflected over shell. Anterior half of mantle margin with a ciliated lobe on inside of inner mantle fold (Figs. 16, 19); posterior half of inner fold of mantle border heavily ciliated (Figs. 17, 20). Pallial glands developed on inner surface at posterior part of presiphonal suture (Fig. 22). Pedal opening long, extending for two-thirds of mantle margin length, anteriorly differentiated into a short, frilled-edge, inhalant region that can be extended beyond shell margin (Fig. 24). Short pre-siphonal suture (Fig. 22) and minute exhalant siphon, present. This “siphon” does not extend beyond shell margin in living specimens. Small papillae on middle mantle fold, present (Figs. 24, 25). Transverse section of anterior and posterior adductor muscles small, ovate, the anterior larger (Figs. 14, 15). Labial palps triangular,

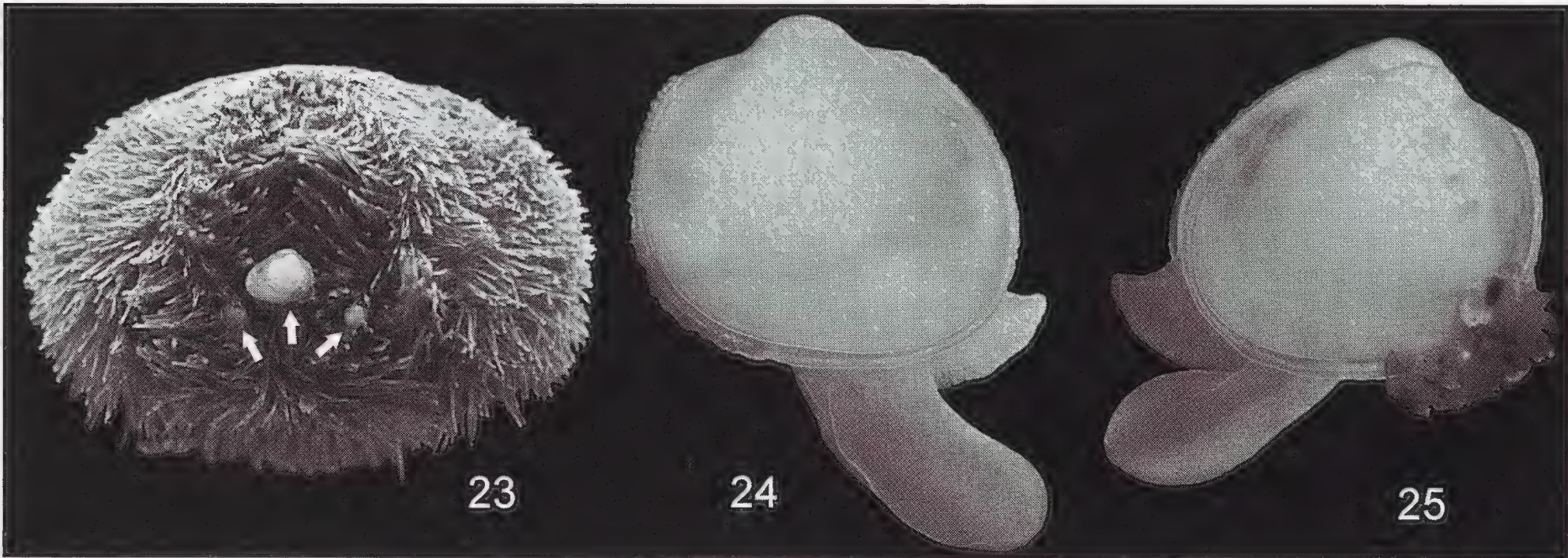
the outer, the larger with five sorting ridges; inner with 2–3 sorting ridges (Figs. 14, 15). Only inner demibranch present; triangular in outline, with up to 50 filaments with very long adfrontal extensions (Figs. 14, 21); not infrequently, adfrontal extensions of contiguous filaments anastomose. Gill axis almost vertical. Ascending lamellae two-thirds length of descending lamellae (Fig. 17). In lower part of gill, adfrontal extensions of descending and ascending parts of several filaments united, connecting outer and inner lamellae (Fig. 32). Upper end of ascending lamellae fused to visceral mass (Fig. 17); behind visceral mass, posterior ends of left and right ascending lamellae fused, and upper edges of descending lamellae fused to inner mantle epithelium (Fig. 18). Foot ciliated, cylindrical, its base able to form a creeping sole, with slightly marked posterior heel (Figs. 14, 15, 25). Byssal groove running mid-ventrally from heel to just behind anterior tip. Byssus gland large, persistent in adults, opening into a relatively long byssus groove, formed by left and right halves. Byssus composed of a single thread at base, distally split into several very thin treads (Fig. 14). Anterior and posterior retractor pedal muscles large, strong; anterior pedal protractor delicate, crossing anterior adductor muscle (Fig. 15); byssus retractor muscle not well defined.

Biological Observations: *Tellimya tehuelcha* lives as an epibiont on the heart urchin *Abatus cavernosus* (Philippi, 1845), particularly in muddy sand, where it was found attached by its byssus exclusively at the urchin’s anal region (Fig. 23). Echinoids of 20.9 to 39.1 mm length were found to host these bivalves (examined range: 4.95–39.1 mm). The bivalve did not show a differential axis of orientation with respect to the echinoid oral-aboral axis. Specimens of *Tellimya tehuelcha* were found all year round on echinoids. These observations, and the fact that no free living specimens were found when sieving the sediments where echinoids were present, support the assumption that *Tellimya tehuelcha* develops its entire life history on *Abatus cavernosus*. The number of bivalves per echinoid varied between 1 and 10 (Fig. 26); when more than one specimen was present on a single host, only one large specimen (over 2 mm length) was present; the remaining specimens usually were juveniles.

A total of 32 specimens (1.25–3.4 mm length) were studied histologically. *Tellimya*



FIGS. 14–22. *Tellimya tehuelcha*, n. sp., anatomy. FIG. 14: Gross anatomy (left side); FIG. 15: Sagittal section; FIGS. 16–22: Transverse sections showing details of demibranch, demibranch fusion, and mantle border. FIG. 16: Anterior section; FIG. 17: Median section; FIG. 18: Posterior section showing fusion of demibranchs and posterior mantle border; FIG. 19: Detail of the anterior portion of the mantle border; FIG. 20: Detail of the median portion of the mantle border; FIG. 21: Detail of demibranch showing the adfrontal extension of filaments and the fusion of ascending lamella to the visceral mass; FIG. 22: Detail of posterior fusion of the mantle border at the level of presiphonal suture and pallial glands. Scale bars: Fig. 15 = 500 μ m; Figs. 16–18 = 200 μ m; Figs. 19, 21, 22 = 300 μ m; Fig. 20 = 150 μ m. Abbreviations - aam: anterior adductor muscle; af: adfrontal extensions of branchial filaments; al: ascending lamella; app: anterior protractor pedis; arp: anterior retractor pedis; bf: branchial fusion; by: byssus; cg: cerebral ganglion; cl: ciliated lobe; dg: digestive gland; dl: descending lamella; g: gill; go: gonad; f: foot; imf: inner mantle fold; l: ligament; lp: labial palp; mmf: middle mantle fold; omf: outer mantle fold; p: periostracum; pam: posterior adductor muscle; pg: pallial glands; prp: posterior retractor pedis; r: rectum.



FIGS. 23–25. *Tellimya tehuelcha*, n. sp., living specimens. FIG. 23: Bivalves on the echinoid (arrows indicate the exact position of the bivalves); FIG. 24: Right side; FIG. 25: Left side.

tehuelcha is a peculiar simultaneous hermaphrodite, with an initial stage of gonadal development when, despite their reduced extension in the visceral mass, male tissues are preponderant, and a subsequent stage when female tissues are dominant (Fig. 27). Specimens at sizes below 1.8–2 mm in length, showed acini containing only early male germ cells: spermatogonia and spermatocytes I, the latter with clear figures corresponding to the first meiotic prophase (Fig. 28). In these specimens, male tissues are localized mainly at the posterodorsal region of the visceral mass.

In specimens larger than 2 mm length, female germ cells progressively become evident (Figs. 27, 29). As vitellogenesis proceeds, a reduced number spermatocytes remain as small groups of germ cells blocked throughout vitellogenesis at the stage of spermatocyte I. Seminal receptacles were not observed. Vitellogenesis starts early, when oocytes reach about 80 μ m diameter (Fig. 30). The diameter of ripe oocytes is about 350–400 μ m (Fig. 31). Gonads in late vitellogenic stage showed male germ cells still at the spermatocyte I stage; specimens

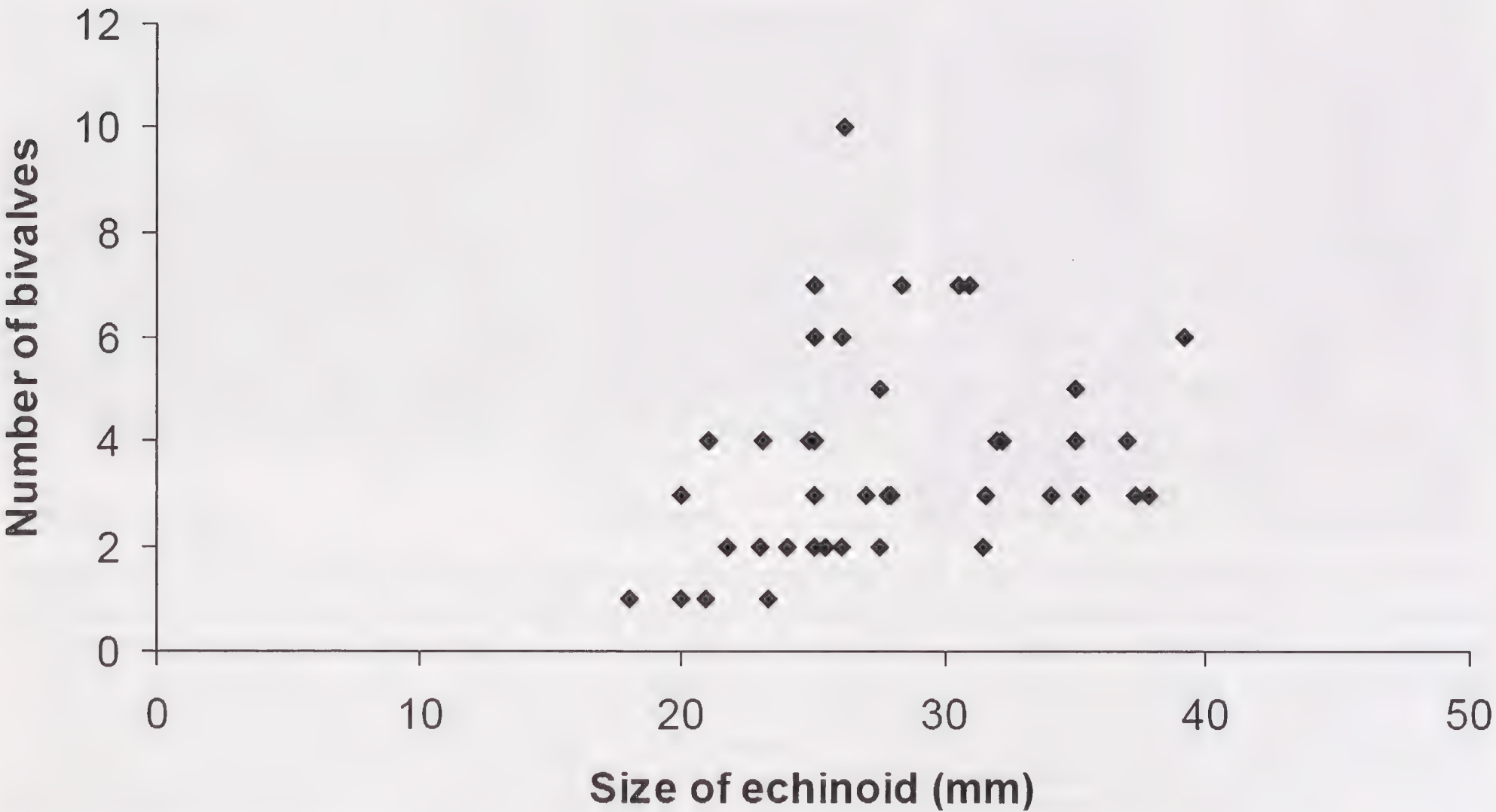
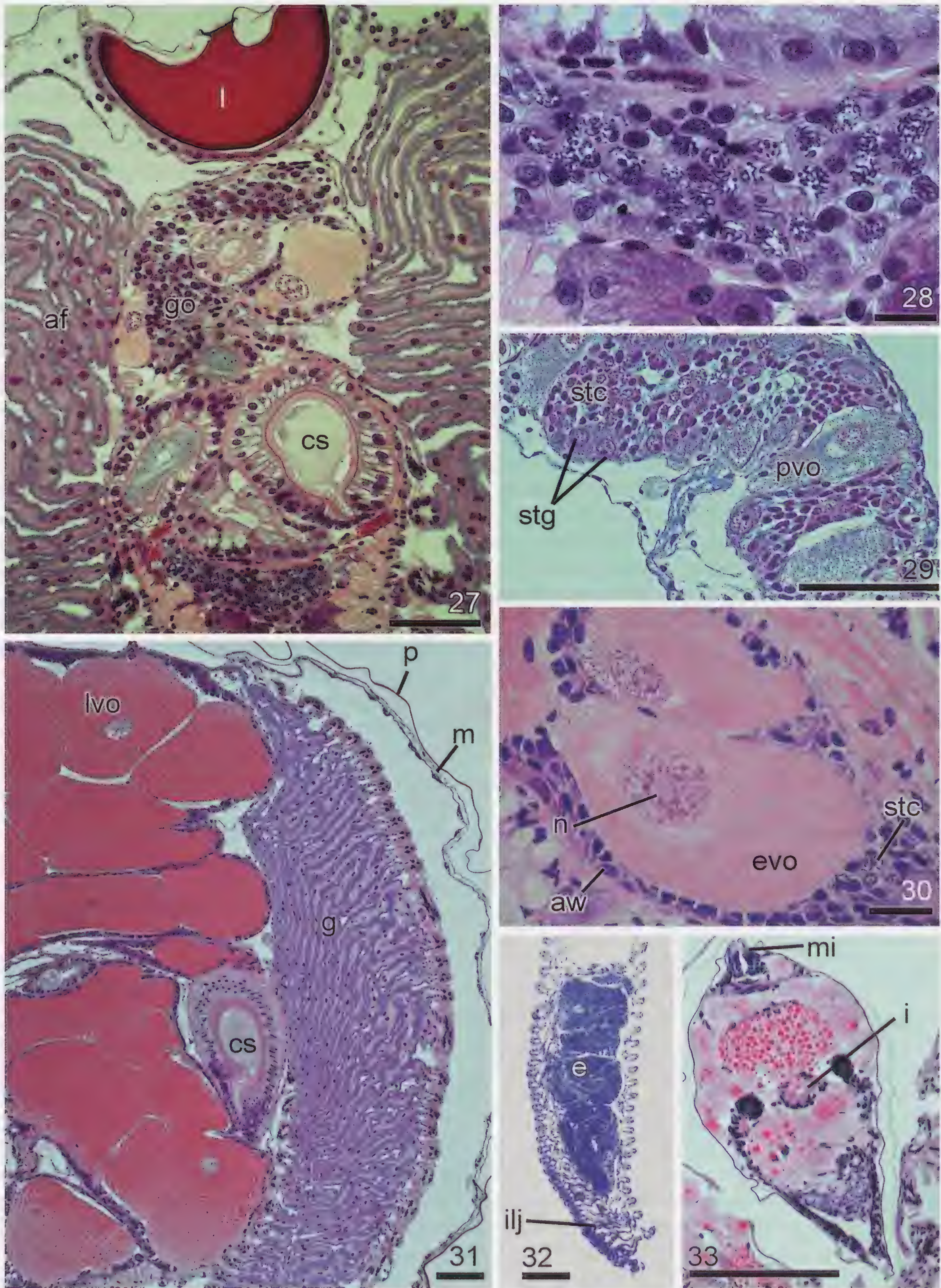


FIG. 26. Relationship between echinoid size and number of bivalves.



FIGS. 27–33. *Tellimya tehuelcha*, n. sp., histological sections of the visceral mass and embryos. FIG. 27: Hermaphrodite gonad of a 2.4 mm length specimen; FIG. 28: Detail of spermatocytes showing characteristic figures of first meiotic prophase; FIG. 29: Detail of spermatocytes I and pre-vitellogenic oocytes; FIG. 30: Detail of early vitellogenic oocytes; FIG. 31: Oocytes in late vitellogenic stage; FIG. 32: Early developing embryos within the inner demibranch; FIG. 33: Late developing embryo. Scale bars: Figs. 27, 31 = 100 μ m; Fig. 28 = 20 μ m; Fig. 29 = 50 μ m; Fig. 30 = 30 μ m; Figs. 32, 33 = 200 μ m. Abbreviations - af: adfrontal extension of branchial filaments; cs: crystalline stylet; e: embryos; evo: early pre-vitellogenic oocyte; aw: acinus wall; g: gill; go: gonad; i: intestine; ilj: interlamellar junction; l: ligament; lvo: late vitellogenic oocytes; m: mantle; mi: mantle isthmus; n: nucleus; p: periostracum; pvo: pre-vitellogenic oocyte; stg: spermatogonia; stc: spermatocytes.

ready to spawn showed few mature sperms intermingled among female acini.

Released ova are retained in both inner demibranchs (Fig. 32), where embryos develop. Up to 33 embryos per demibranch were found in a specimen of 3.3 mm in length. Brooding embryos remain free within the branchial space of the maternal individuals until complete their development and being released as miniature juveniles (about 700 μm length) (Fig. 33).

DISCUSSION

Tellimya tehuelcha resembles in general shell shape and gross anatomy *Tellimya ferruginosa*, the type species of the genus; both species have only one (the inner) demibranch, with anastomosing gill filaments; mantle margin, with papillae, not covering the outer shell surface; foot with anterior and posterior retractor muscles and pedal protractor, but lacking of byssus retractor muscle; absence of seminal receptacles; shell with posteriorly displaced beaks; hinge plate interrupted below beaks, with only one, anterior, obliquely directed cardinal tooth in the right valve, and the left valve with only a long, slender tooth parallel to the dorsal margin, anterior to the beak. These characters support the cogeneric status of the two species.

Tellimya tehuelcha differs from *T. ferruginosa* in having a less projecting anterior end and more markedly truncate posterior margin, resulting, in adult specimens, in a markedly trapezoidal (instead ovate) shell outline. The protoconch is larger in *T. tehuelcha* (about 700 μm) than in *T. ferruginosa* (410 μm). *Tellimya ferruginosa* has, in addition to papillae on the mantle margin, two "tentacles", dorsal and ventral to the exhalant siphon, which are absent in *T. tehuelcha*. Deroux (1961) reported for *T. ferruginosa* an external part of the ligament, which is not present in *T. tehuelcha*, where the ligament is completely internal. According to Deroux (1961), *T. ferruginosa* has the ascending lamella of inner demibranch nearly as long as the descending lamella, a condition quite different from that observed in *T. tehuelcha*.

Tellimya tenella (Lovén, 1846) has a flat, oval shell, with convex dorsal margin, faint microscopic radial striae, and very narrow hinge plate. This species, as *T. ferruginosa*, has a smaller protoconch than *T. tehuelcha* (about 420 μm in diameter) and two large siphonal tentacles, one above and the other below the exhalant siphon.

Tellimya semirubra (Gaglioli, 1992) differs from *T. tehuelcha* in having a triangular shell outline, and *Tellimya vitrea vitrea* (Hedley, 1907), *Tellimya benthicola* (Dell, 1956), *Tellimya reinga* (Crozier, 1966), and *Tellimya vitrea aupouria* Ponder, 1968, differ from *T. tehuelcha* in having elongate oval shell outlines, sculptured with radial riblets. *Tellimya vitrea aupouria* has in addition a protoconch smaller than that in *T. tehuelcha* (about 300 μm in diameter) and the ascending lamella of inner demibranch are nearly as long as descending lamella.

As it has been reported by Oldfield (1961) for *Tellimya ferruginosa* and Jespersen et al. (2007) for *Epilepton clarkiae* (Clark, 1852), the byssus gland of *Tellimya tehuelcha* is divided in two parts by a central lamella, and differs from that described for *Montacuta substriata*, in which the glandular duct open to a radial structure formed by numerous lamellae (Oldfield, 1961).

Mode of Life

The species of *Tellimya* for which their habitat is known are frequently associated to irregular echinoids. In this regard, the mode of life of *Tellimya tehuelcha* is similar to that reported for the small specimens of *T. ferruginosa*, which live attached to the ventral or subanal spines of the echinoids *Echinocardium cordatum* (Pennant, 1777), *E. flavescens* (Müller, 1776), *E. pennatifidum* Norman, 1868, and *Spatangus purpureus* (Müller, 1776) (Pophan, 1940; Oldfield, 1961; Gage, 1966a). However, larger specimens of *T. ferruginosa* occur free in the burrow produced by the echinoid. This fact is related to the absence of byssus gland in larger specimens of *T. ferruginosa* (Pérès, 1937; Gage, 1966a). On the contrary, in *T. tehuelcha* the byssus gland is persistent in adults, and adult specimens also appear attached to the host. Pérès (1937) reported that usually two or three specimens of *T. ferruginosa* are found per echinoid, with a maximum of five or six. Marshall (1891) reported up to 18 bivalves per echinoid, but being only one of them "mature". These characteristics on the mode of life are similar to those observed in *T. tehuelcha*, in which up to ten attached bivalves per echinoid were found, with only one of them full sized.

Tellimya vitrea aupouria was reported as living on the interambulacrum five of the irregular echinoid *Brissus gigas* Fell, 1947 (Ponder, 1968), and *Tellimya tenella* as living free, in fine sediments (Ockelman, 1965) or "on *Brissopsis lyrifera* (Forbes) (?)" (Lovén, 1848).

Reproductive Biology

According to Oldfield (1961), *Tellimya ferruginosa* is a protogynous, consecutive hermaphrodite, with the gonad developing first entirely as female, undergone a short transition period after the ova are shed, and then become entirely male. Jespersen et al. (2004) stated that the montacutid *Brachiomya stigmatica* Jespersen, Lützen & Nielsen, 2004 might be considered either as a protogynic or alternate hermaphrodite. Our findings for *Tellimya tehuelcha* indicate that the species should be considered a protandric hermaphrodite.

The development of *Tellimya tehuelcha* is entirely lecithotrophic and occurs within the branchial space of the parental specimens; consequently, the free-living larval stage is suppressed. According to Pophan (1940) and Gage (1966b), *Tellimya ferruginosa* retains early larvae (up to a size of about 140 µm diameter) in the demibranch, which are later released, following a planktonic life period until they metamorphose and settle, when reaching about 410 µm diameter. This is consistent with that described for *Tellimya tenella*, in which shelled embryos (of 127–137 µm in diameter) were found in the demibranch of parental specimens, and free veligers of up to 420 µm in diameter were found in the plankton (Ockelman, 1965). The study of the reproductive biology of *Tellimya tehuelcha* is currently in progress.

Geographic Distribution

At the present state of knowledge of the genus, the distribution of *Tellimya* shows as a patchy global pattern. Species of the genus were reported from the Northern Hemisphere in the northeast Atlantic (*Tellimya ferruginosa*, *Tellimya tenella*, *T. voeringi*) and Mediterranean Sea (*Tellimya ferruginosa*, *Tellimya semirubra*); other reports also come from the Southern Hemisphere in Australia (*Tellimya vitrea vitrea*), and New Zealand (*Tellimya vitrea aupouria*, *Tellimya benthicola*, *Tellimya reinga*).

The original illustration of *M. cylindracea* Smith, 1885, a species described from the “North Atlantic deep waters”, shows a hinge plate similar to that of *Tellimya*. According to Serge Gofas (personal communication), *M. cylindracea* may be a synonym of *T. ferruginosa*.

Four species from the sub-Antarctic and Antarctic waters have been described under *Tellimya* (i.e., *T. antarctica* Smith, 1907, *T.*

minima Thiele, 1912, *T. ovalis* Thiele, 1912, and *T. flavida* Preston, 1916), but all were subsequently reallocated by Dell (1964) into *Mysella* Angas, 1877, and *Rochefortia* Velain, 1877. Thus, the new species described here provides new evidence on the occurrence of this genus in the Southern Hemisphere, and represents the first record not only for the Magellan Region but also for South America.

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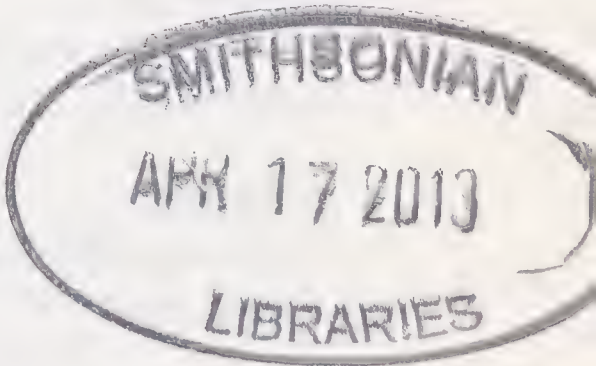
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THE MOLLUSCAN GENERA *MACTRELLA* AND *MACTRINULA*: TAXONOMIC REVISION AND REDESCRIPTION OF TYPE SPECIES

Javier H. Signorelli

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ABSTRACT

The genera *Mactrella* and *Macrinula*, both described by Gray in 1853, have generally been considered synonyms. Additionally, five species had been included in *Mactrella* were later placed in the genus *Mactrellona* Marks, 1951. The diagnosis of type species of these genera, as well as the study of their type material, have clarified the taxonomic status of the three related genera. The genus *Micromactra* Dall, 1894, is added to the synonymy of *Mactrella*. The complete shell morphology of type species *Mactrella striatula* and *Macrinula plicataria*, both introduced by Linnaeus, 1767, is described. An updated synonymy list of each species is provided. In addition, an expanded geographical distribution of the genus *Mactrella* is given. Illustrations of *Mactrellona alata* (Spengler, 1802), *Mactrellona exoleta* (Gray, 1837), *Mactrellona clisia* (Dall, 1915), *Mactrella californica* (Conrad, 1837), *Harvella elegans* (G. B. Sowerby I, 1825), and *Mactrella janeiroensis* (E. A. Smith, 1915) are included.

Key words: Bivalves, Mactrinae, taxonomy, Gray, Linnaeus, *Mactra striatula*, *Mactra plicataria*.

INTRODUCTION

The family Mactridae first appeared in the Lower Cretaceous (Saul, 1973; Skelton & Benton, 1993) of North America. One key morphological character that defines mactrids is an inverted V-shaped tooth in the left valve formed by the fusion of two cardinal teeth (Keen, 1969). The anterior and posterior lateral teeth have, in general, only one cusp. Several authors have studied such anatomical characters as ctenidial morphology, labial palp fusion, siphons, stomach, and shell ultrastructure, to better understand the morphology and its variation within the group (Atkins, 1937; Yonge, 1948; Purchon, 1960; Stasek, 1963; Taylor, 1973). The Mactridae includes about 90 generic-level entities, several of which are taxonomically unresolved.

During the first half of the nineteenth century, Gray (1837) split the genus *Mactra* into five sections. Two of them were “*Mactra* B,” in which he placed *Mactra striatula* Linnaeus, 1767, and “*Mactra* C,” which included *Mactra plicataria* Linnaeus, 1767. All sections mentioned by Gray in 1837 were formalized in 1853 when he introduced the genera *Mactrella* and *Macrinula* for the *Mactra* B and C

groups, respectively. The original composition included only the two Linnean species mentioned above. Throughout the twentieth century, *Mactrella* and *Macrinula* have been generally considered synonyms (e.g., Marks, 1951; Keen, 1969). Dall (1915) included five species in the genus *Mactrella*, two from the Atlantic and three from the Pacific Ocean. Marks (1951) later said that these should be assigned to his new genus *Mactrellona*, with type species *Mactra alata* Spengler, 1802. He based the description of this new genus on the diagnosis of *Mactrella* given by Gray, which actually fits with the morphology *M. alata* better than it does that of *M. striatula*. However, the type species designated by an author must be accepted assuming the correct identification of the species proposed (ICZN, 1945, Opinion 168). For this reason, Marks (1951) introduced a new genus to group the species *M. alata* and its congeners.

During an ongoing revision of western Atlantic Mactridae (i.e., Signorelli & Scarabino, 2010; Signorelli & Pastorino, 2011, 2012a, b), it became necessary to undertake a taxonomic revision and redescription of the type species of genera *Mactrella* and *Macrinula*, the object of the present work.

MATERIALS AND METHODS

Examined type material and additional specimens illustrated in this paper are deposited in the following institutions: American Museum of Natural History (AMNH), New York; Linnean Society of London (LSL), London; Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-In), Buenos Aires; The Natural History Museum (NHMUK), London; Natural History Museum (NHMW), Vienna. Digital pictures were made with a Nikon D5000 equipped with a 60-mm Nikkor micro lens. The hinge was described with the method developed by Bernard and Munier Chalmas (according to Cox, 1969), in which Arabic numbers were used to designate the cardinal teeth and Roman numbers for the lateral teeth. Additionally, all teeth in a right valve were assigned odd numbers, and all teeth in a left valve were assigned even numbers.

SYSTEMATIC RESULTS

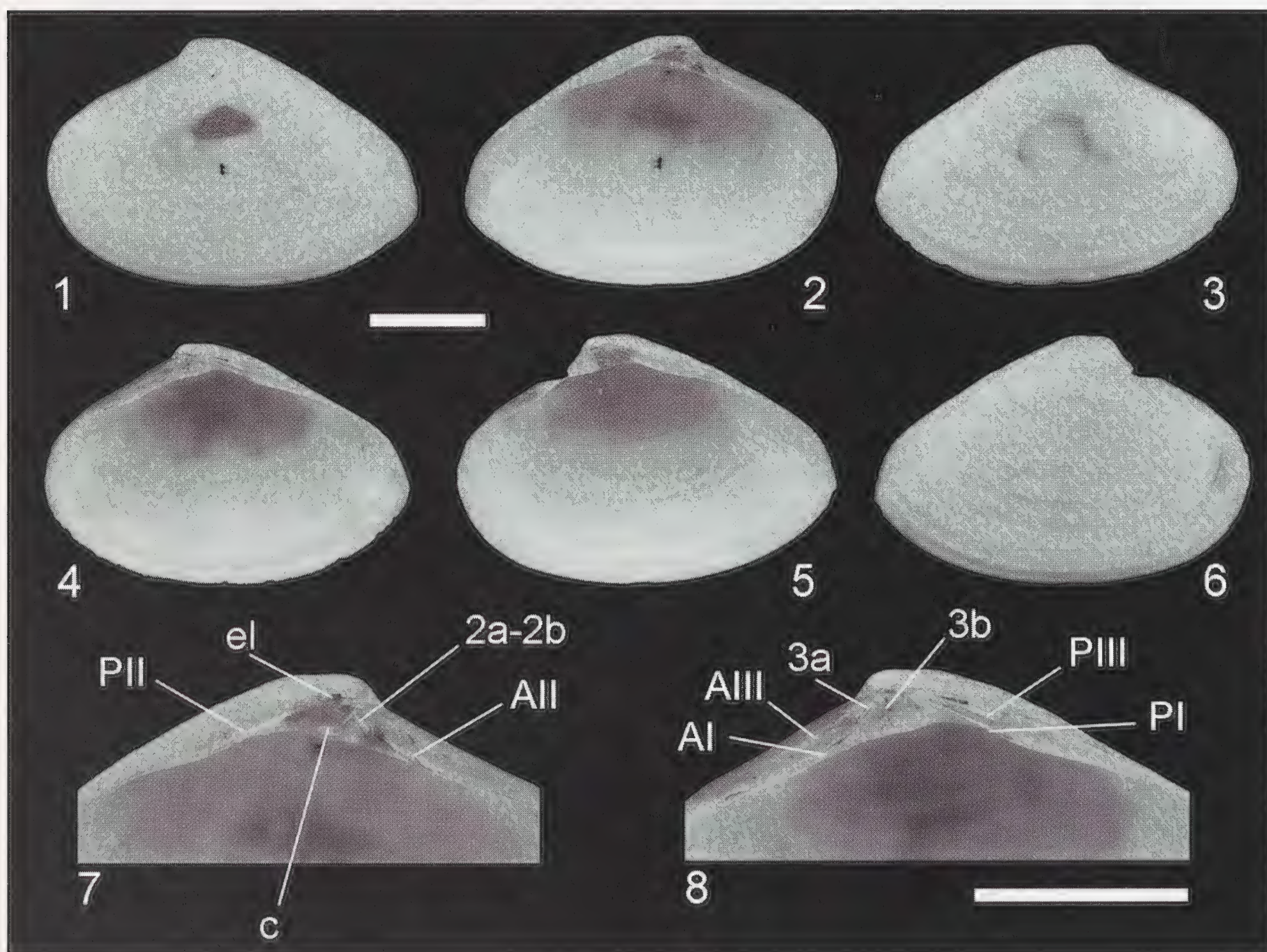
Order Cardiida Férussac, 1822
Superfamily Mactroidea Lamarck, 1809
Family Mactridae Lamarck, 1809

Genus *Mactrella* Gray, 1853.
[*Micromactra* Dall, 1894: 40; type species: *Mactra californica* Conrad, 1837: 240; monotypy]

Type species: Mactra striatula Linnaeus, 1767: 1125; monotypy.

Diagnosis: Shell trigonal to oval, beaks and umbones with concentric ribs; moderately fragile, pallial sinus deep, broad.

Distribution: West North America, East Central and South America, Europe.



FIGS. 1–8. Shells of *Mactrella striatula* (Linnaeus, 1767), syntypes LSL 78. FIGS. 1–6: General aspect; FIGS. 7, 8: Detail of hinge plate, el: external ligament, c: chondrophore. Scale bars = 1 cm.

Mactrella striatula (Linnaeus, 1767)
(Figs. 1–8)

Macra striatula Linnaeus, 1767: 1125; Gmelin, 1791: 3257; Spengler, 1802: 99; Hanley, 1855: 55, pl. 2, fig. 3; Mörch, 1870: 122.

Mactrella striatula Linnaeus. Gray, 1853: 41.

Mactrinula striatula Linnaeus. H. Adams & A. Adams, 1856: 377; Conrad, 1868: 35.

Macra (Mactrinula) striatula Linnaeus. Lamy, 1917: 270.

Diagnosis: Shell trigonal, with low commarginal ribs in the umbonal area; posterior end with a well-defined carina; umbos moderately inflated; pallial sinus deep, rounded.

Description: Shell trigonal, elongate, inflated, inequilateral, fragile; umbones moderately inflated, with commarginal striae, especially laterally, becoming smoother toward the ventral shell margin; anterior dorsal margin straight, longer than posterior margin; anterior end rounded; ventral margin curved; posterior carina defined by a line from the umbo to the posterior end, well marked, forming a striated, depressed area; posterior end low. Internally white, pellucid in juveniles; hinge plate with one anterior and one posterior (AII & PII) lateral teeth in the left valve, the anterior longer, inverted V-shaped cardinal tooth fragile formed by two single teeth, the posterior longer; right valve with two anterior (AI & AIII) and two posterior (PI & PIII) lateral teeth, anterior cardinal tooth (3a) oriented in the same axis as anterior ventral lateral (AI); posterior cardinal vertically oriented; internal ligament placed in a well-developed chondrophore, but not enlarged beyond the hinge plate; pallial sinus rounded, wide, deep, about half of shell length.

Type Material: LSL 78.

Type Locality: “in Mare Mediterraneo?”

Distribution: Europe, Mediterranean Sea.

Remarks: The nominal species *Macra laevis* Chemnitz, 1782 (a rejected work; ICZN, 1944, Opinion 184); *Macra subplicata* Lamarck, 1818 (*non M. subplicata* Lamarck of W. Wood, 1828: pl. 1, *Macra* fig. 6) and *Macra spengleri* Linnaeus of Born, 1780 (*non* Linnaeus, 1767) have been regarded as synonyms of *Mactrella striatula* Linnaeus (e.g.,

Gmelin, 1791, Reeve, 1854; Lamy, 1917). However, after analysis of all type material, as well as the Born collection, three distinguishable species can be recognized.

The original illustration of Chemnitz of *Macra laevis* (1782: 214, pl. 21, figs. 205, 206) coincides with the type material of *Macra subplicata* Lamarck (Figs. 9, 10; Figs. 11–17), but clearly differs from the types of *Macra striatula* Linnaeus deposited at LSL. Finally, the studied material from the Born collection (Figs. 18–21) clearly matches the types of *Mactrellona alata* (Spengler, 1802) (Figs. 34–36). The type locality given by Linnaeus was the Mediterranean Sea, whereas the type localities mentioned by Chemnitz and Lamarck were “Indian Ocean.” *Macra dolabrata* Reeve, 1854, *ex Deshayes* ms, considered a synonym of *M. striatula sensu* Huber (2010), is, in fact, *Macra subplicata* Lamarck. The morphology of the type material described by Reeve (NHMUK 1996.444) coincides with the characters observed in the Lamarck species.

Genus *Mactrinula* Gray, 1853

[= *Papyrina* Mörch, 1853: 4, *Macra plicataria* Linnaeus, 1767, subsequent designation by Keen, 1969: N598]

Type species: *Macra plicataria* Linnaeus, 1767: 1125, monotypy.

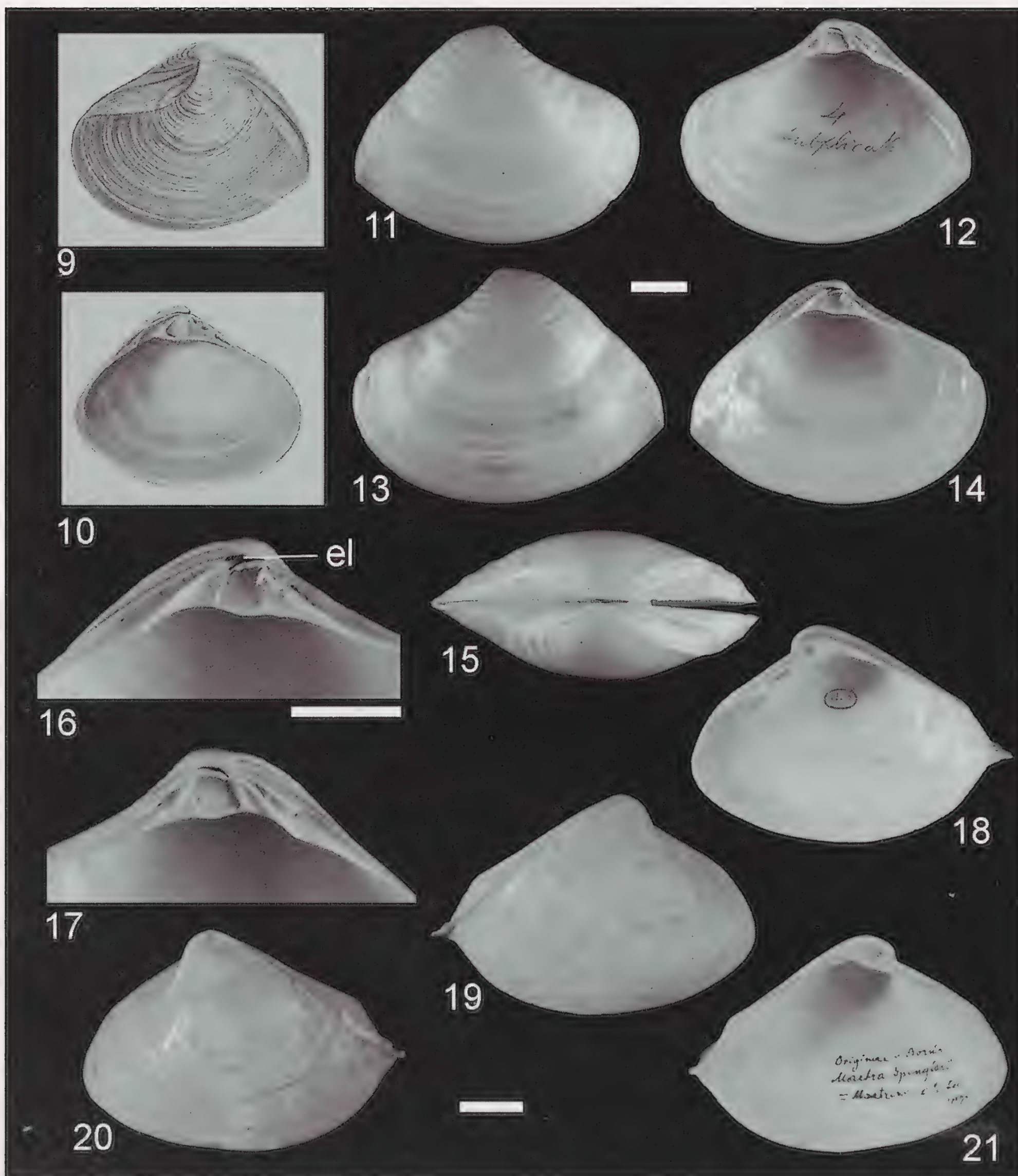
Diagnosis: Valves concentrically plicate, inequilateral; posterior slope set off by ridge; resilifer large and narrow, hinge teeth not concentrated, cardinal in left valve reinforced by apophysis with accessory lamella in front.

Distribution: Indian and Pacific oceans.

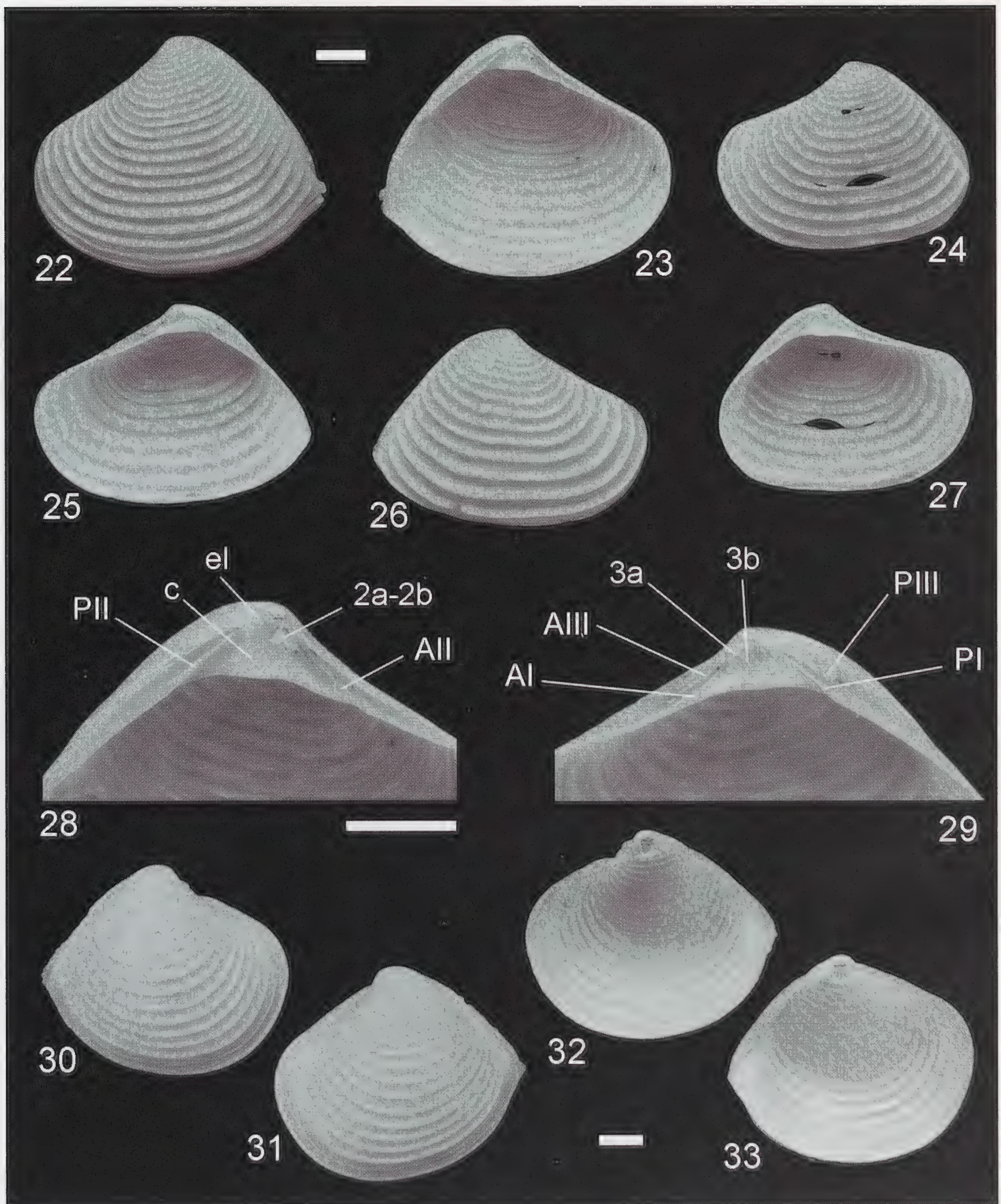
Mactrinula plicataria Linnaeus, 1767
(Figs. 22–29)

Macra plicataria Linnaeus, 1767: 1125; Chemnitz, 1782: 213, pl. 20, figs. 202–204; Gmelin, 1791: 3257; Spengler, 1802: 98; Lamarck, 1818: 476; Bory de Saint Vincent, 1827, in Bruguière et al., 1791–1827: 151; Deshayes, 1830: 396; Deshayes & Milne Edwards, 1835, 102; Gray, 1837: 372, fig. 31; Hanley, 1842: 30; Reeve, 1854: fig. 26; Mörch, 1870: 122; Weinkauff, 1884, in Küster & Weinkauff, 1841–1884: 7, pl. 2, figs. 4–6; Hedley, 1910: 351; Lamy, 1914: 134.

Mactrinula plicaria Gray, 1853: 41, misspelling of *M. plicataria* Linnaeus.



FIGS. 9–21. Shells of *Mactra* spp. FIGS. 9, 10: *Mactra laevis* Chemnitz, 1782 (work rejected), original illustration; FIGS. 11–17: *Mactra subplicata* Lamarck, 1818, syntype MNHN 24189, el: external ligament. Scale bar = 1 cm; FIGS. 18–21: *Mactra spengleri* Born, 1780 (*non* Linnaeus, 1767), original material from Born collection, NHMW 14050. Scale bar = 2 cm.



FIGS. 22–33. Shells of Mactridae. FIGS. 22–29: *Mactrinula plicataria* (Linnaeus, 1767), syntypes LSL 77; FIGS. 22–27: General aspect; FIGS. 28, 29: Detail of hinge plate, el: external ligament, c: chondrophore; FIGS. 30–33: *Harvella elegans* (Sowerby, 1825), syntypes deposited at NHMUK (without number). Scale bars = 2 cm.

Mactrinula plicataria Linnaeus. H. Adams & A. Adams, 1856: 377, pl. 99, fig. 2, 2a; Chenu, 1862: 55, fig. 226; Conrad, 1868: 35; Melvill & Standen, 1907: 828.

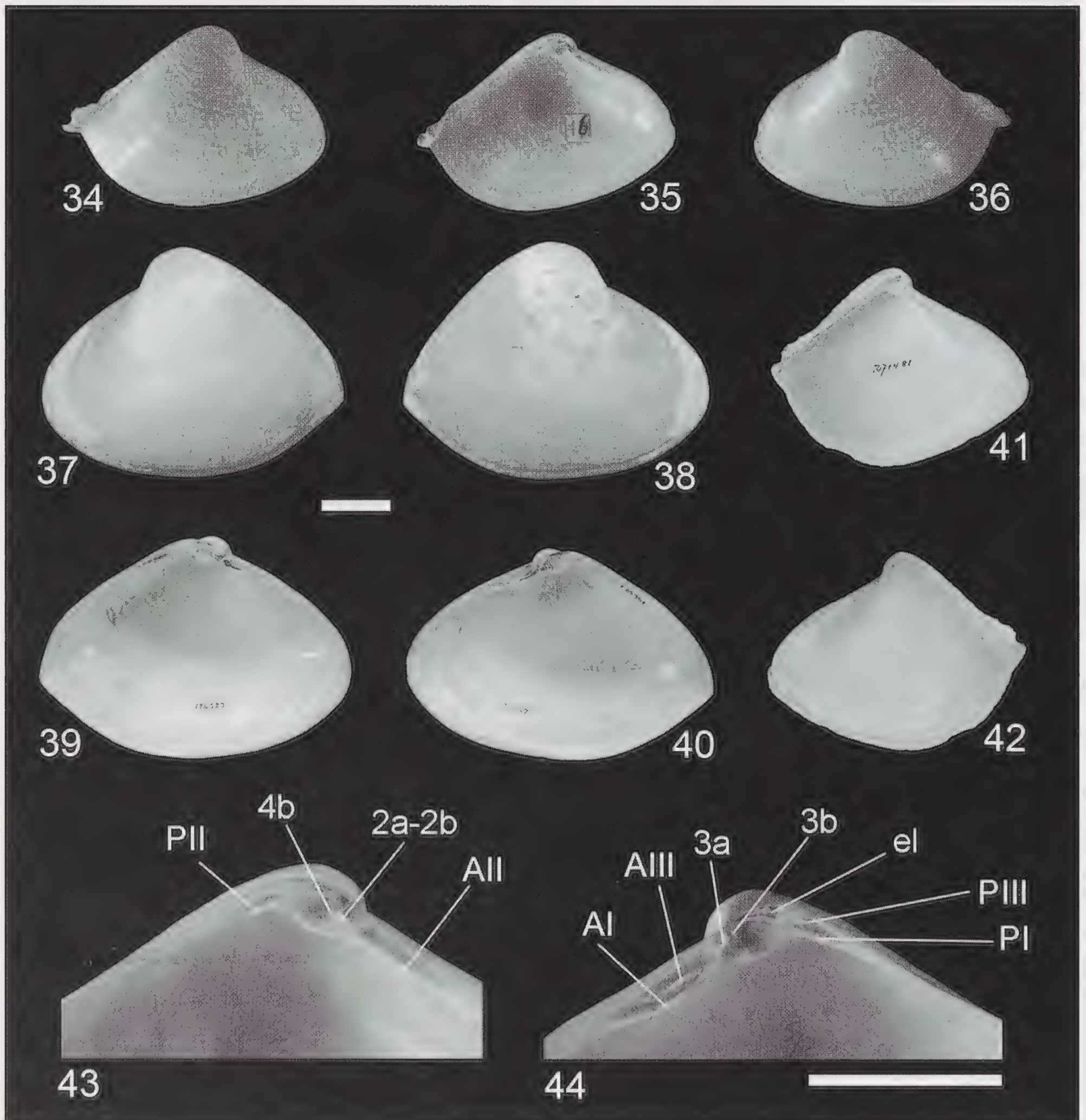
Mactra (Mactrinula) plicataria Linnaeus. Tryon, 1884: 157, pl. 109, fig. 1; E. A. Smith, 1885: 57; 1914: 117; Lamy, 1917: 268; Keen, 1969: N598, figs. E92–94.

Mactrella plicataria Linnaeus. Dall, 1895: 211; 1898: 877.

Harvella plicataria Linnaeus. Abbott & Dance, 1986: 335; Dharma, 1992: 92, pl. 24, fig. 4.

Diagnosis: Shell trigonal, fragile with external surface plicate; posterior end defined by a keel-like carina.

Description: Shell thin and fragile, trigonal, elongate, inequilateral, external surface with com-marginal ridges; anterior margin round, mod-



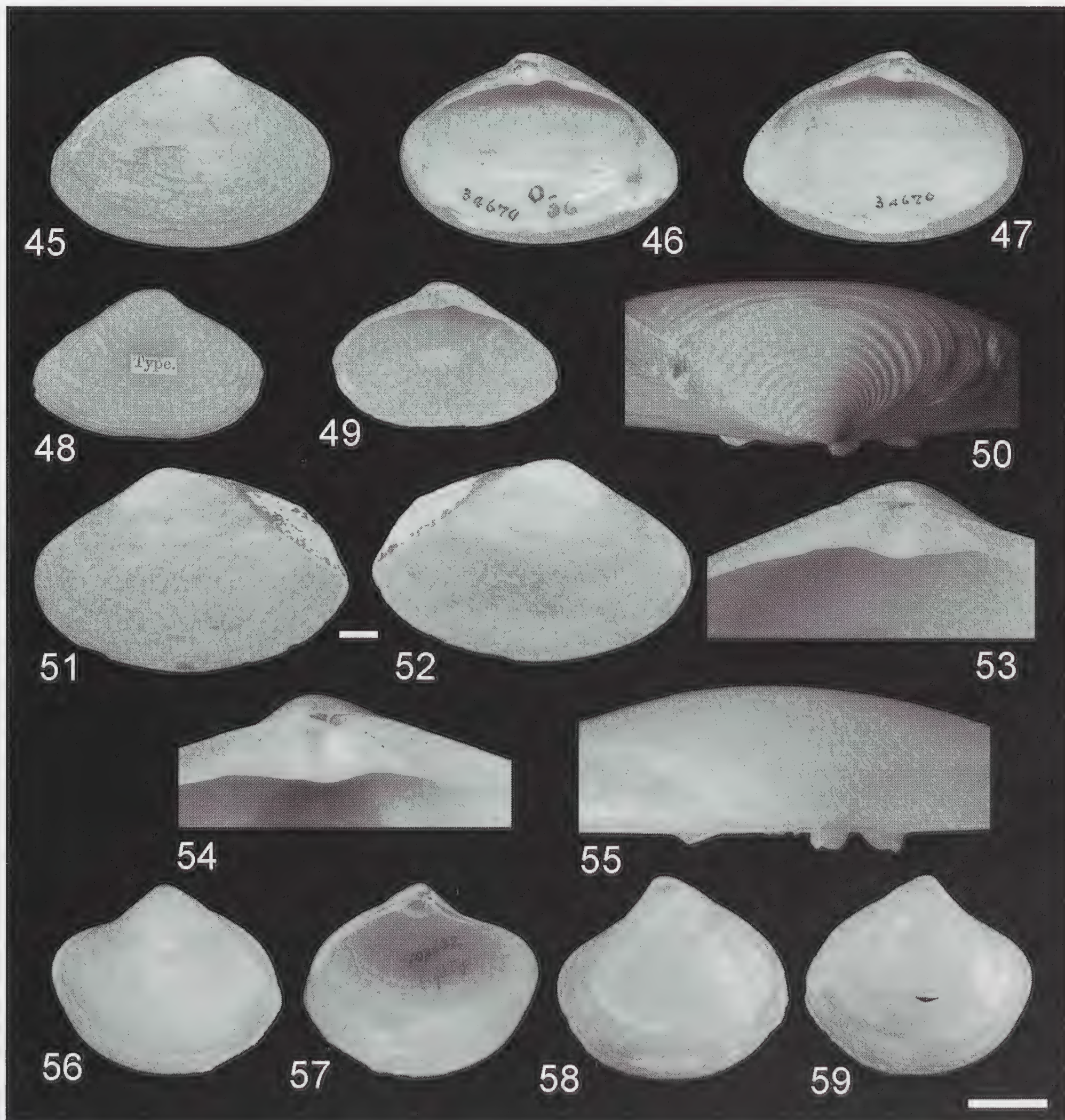
FIGS. 34–44. Shells of *Mactrellona* spp. FIGS. 34–36: *Mactrellona alata* Spengler (Spengler, 1802) holotype, ZMUC unnumbered); FIGS. 37–40: *Mactrellona clisia* (Dall, 1915) holotype, USNM 271481; FIGS. 41, 42: *Mactrellona exoleta* (Gray, 1837), syntype, NHMUK 196327; FIGS. 43, 44: Detail of hinge plate of *M. alata*, el: external ligament. Scale bars = 2 cm.

erately concave, longer than posterior margin; anterior end rounded, posterior end defined by a keel-like carina on which the concentric lines are closely spaced; umbones small, prosogyrous; escutcheon deep; periostracum brownish; internally pellucid with external ornamentation visible due to the shell thinness; left hinge with inverted V-shaped cardinal tooth (2a–2b), the single tooth 2a longer, one anterior and one

posterior lateral tooth (All & PII) elongate, with the dorsal ends close to cardinals; right hinge with two anterior and two posterior lateral teeth, equal in size and shape and less elongate than left laterals, pallial sinus moderate, round.

Type Material: LSL 77.

Type Locality: Java Sea, Indonesia.



FIGS. 45–59. Shells of Mactridae. FIGS. 45–47: *Mactrella californica* (Conrad, 1837), AMNH (34670); FIGS. 48–50: *Mactrella janeiroensis* (Smith, 1915), syntype, NHMUK 1915.4.18.489; FIGS. 51–55: *Standella fragilis* (Gmelin, 1791), AMNH 34063; FIGS. 56–59: *Trinitasia iheringi* (Dall, 1897); FIGS. 56, 57: Holotype of *Mactrella iheringi* (USNM 107632); FIGS. 58, 59: *Mulinia kempfi* Cauquoin, 1969, two syntypes (MNHN unnumbered). Scale bars = 1 cm.

Distribution: Indian and Pacific oceans, Malaysia, Thailand, Indonesia, India, Australia.

Remarks: Although the generic position of *Mactrinula plicataria* has varied among authors (e.g., Lamy, 1917; Abbott & Dance, 1986), this species was proposed as the type species of *Mactrinula* by Gray (1853). The inclusion of *M. plicataria* in the genus *Harvella*, as some authors have proposed, is not accepted. Although the morphology of the external surface could suggest this assignment, the hinge morphology observed in this Linnean species significantly differs from that of *Harvella elegans* G. B. Sowerby I, 1825, type species of *Harvella* (Figs. 30–33). The lateral teeth are longer, and the resilifer is less ventrally projecting in *Mactrinula*. In addition, the anterior dorsal edge is rounded and dorsally prolonged in *Harvella*, whereas in *Mactrinula*, it is straight and inclined anteriorly. *Mactra subplicata* Lamarck of W. Wood (1828), *non M. subplicata* Lamarck, 1818, was considered a synonym of *M. plicataria* (Lamy, 1917); however, the illustration in Wood is extremely rudimentary and does not allow a taxonomic conclusion to be drawn.

DISCUSSION

A study of the type species of *Mactrinula* and *Mactrella* reveals the presence of two different and valid groups. A complete comparison of diagnostic characters of the genera treated here is given in Table 1. The introduction of *Mactrellona* Marks clarifies the taxonomic status of *Mactrella*. Marks (1951) described his new genus to provide a name for a group of species that includes *M. alata* Spengler, 1802, *M. clisia* Dall, 1915, and *M. exoleta* Gray, 1837 (Figs. 34–44), previously placed in *Mactrella*. The congeneric relationship of *Mactra plicataria* (type species of *Mactrinula*) and *Mactra striatula* (type species of *Mactrella*) mentioned by Marks (1951) is incorrect. The shell morphology of the type specimens reveals two distinguishable genera (Figs. 1–8 vs. Figs. 22–29). The external ornamentation of *Mactrinula plicataria* plus the hinge morphology suggest a generic position different from the species included in *Mactrella*. In addition, Marks (1951) erroneously cited both taxa from the Indo-Pacific region; however, *Mactra striatula* Linnaeus was described from European waters.

The inclusion of species closely related to *Mactra alata* Spengler in the genus *Mactrella*

was based on the diagnosis given by Gray (1853). The characters of *M. striatula* mentioned by Gray fit better with those observed in *M. alata* (Marks, 1951). In addition, in the “*Mactra* B” section of Gray (1837), he mentioned *M. carinata* Lamarck, 1818, as synonymous with *M. striatula*. However, this nominal species is, in fact, the same as *Mactrellona alata* (e.g., Dall, 1894; Lamy, 1917). Evidently, Gray misidentified the specimens considered by him as *M. striatula*. These facts allowed Marks (1951) to introduce the new genus *Mactrellona* for the *M. alata* group and to keep *M. striatula* as type species of *Mactrella*. Marks based his decision on Opinion 168 (ICZN, 1945), which considered that a species designed as type of a genus must be accepted assuming that the original author of a genus correctly identified the species assigned by him in the absence of evidence to the contrary.

The genus *Micromactra* described as a section of *Mactra* by Dall (1894) is here added to the synonymy of *Mactrella*. This genus was included in the synonymy of *Mactrotoma* Dall, 1894, by Coan & Valentich-Scott (2012). In addition, Petit (2012) noted that the genus *Standella* Gray, 1853, is a senior objective synonym of *Mactrotoma* because they have the same type species (*M. fragilis* Gmelin, 1791). The type species of *Standella*, *Micromactra*, and *Mactrella* all have commarginal ribs on their umbones. However, *M. striatula* and *M. californica* present additional similarities that suggest a congeneric condition (Figs. 45–47). These are a trigonal shell with concentric wrinkles on the posterior umbonal area, a hinge plate with elongate lateral teeth not concentrated in the cardinal area, and a sharply rounded posterior end without a siphonal gape. These similarities were pointed out by Hanley (1855). However, *M. fragilis* is characterized by a less inflated and oval shell with a smooth posterior umbonal external surface, and the hinge plate is fragile, with short lateral teeth close to the cardinals. The posterior end of *M. fragilis* is truncate and gaping (Figs. 51–55).

The synonymy, proposed here, expands the geographic distribution of *Mactrella* along the western Atlantic Ocean from the southern coast of the United States to northern Patagonia in Argentina and to the Pacific coast of North America (Abbott, 1986; Signorelli & Pastorino, 2012a). *Mactrella californica* (Conrad, 1837) and *Mactrella janeiroensis* (Smith, 1915) are new combinations of two valid species (Figs. 48–50). The genus *Papyrina* introduced by Mörch in April 1853 grouped several species

TABLE 1. Diagnostic characters to differentiate the treated valid genera.

Genus	Type species	Shell shape	Hinge plate	Sculpture	Revised species included
<i>Harvella</i> Gray, 1853	<i>M. elegans</i> G. B. Sowerby I, 1825	Ovate, thin, translucent, with a posterior keel	Lateral teeth close to the cardinals, external ligament separated from resilium by a ridge	With heavy commarginal undulations	<i>Harvella elegans</i> (G. B Sowerby I, 1825)
<i>Mactrella</i> Gray, 1853 (= <i>Micromactra</i> Dall, 1894, type <i>M. californica</i> Conrad, 1837)	<i>M. striatula</i> Linnaeus, 1767	Trigonal shell, posterior end sharply rounded without siphonal gap	Hinge plate with elongate lateral teeth not concentrated on the cardinal area	With concentric wrinkles on the post umbonal area of the external surface	<i>Mactrella striatula</i> (Linnaeus, 1767) <i>Mactrella californica</i> (Conrad, 1837) <i>Mactrella janeiroensis</i> (Smith, 1915)
<i>Mactrellona</i> Marks, 1951	<i>M. alata</i> Spengler, 1802	Large with a posterior area defined by keel	Hinge plate inclined anteriorly, with short lateral teeth	With commarginal striae, external surface smooth	<i>Mactrellona alata</i> (Spengler, 1802) <i>Mactrellona exoleta</i> (Gray, 1837) <i>Mactrellona clisia</i> (Dall, 1915)
<i>Mactrinula</i> Gray, 1853	<i>M. plicataria</i> Linnaeus, 1767	Trigonal, elongate, thin, inequilateral; external ornamentation evidenced due to shell thinness	Hinge teeth not concentrated, cardinal in left valve reinforced by apophysis with accessory lamella in front; resilifer large, narrow	Widely spaced concentrically plicate; posteriorly, with a pronounced keel.	<i>Mactrinula plicataria</i> (Linnaeus, 1767)
<i>Standella</i> Gray 1853 (= <i>Mactrotoma</i> Dall, 1894)	<i>M. fragilis</i> Gray (= <i>M. fragilis</i> Gmelin, 1791)	Trigonal to oval, inequilateral, posterior end truncate and gaped, posterior dorsal area defined by a conspicuous line	Fragile with short lateral teeth close to the cardinals	With the post umbonal external surface totally smooth	<i>Standella fragilis</i> (Gmelin, 1791)
<i>Trinitasia</i> Maury, 1928	<i>Thyasira sanctiandreae</i> Maury, 1925	Shell trigonal to subcircular, lunule well defined, anterior end round and high	Lateral teeth short, close to cardinals, anterior ones almost fused with anterior cardinal tooth, accessory lamella strong	External surface smooth	<i>Trinitasia iheringi</i> (Dall, 1897)

that currently represent more than one genus. He included both *M. striatula* and *M. plicataria* Linnaeus in this genus. The type species of *Papyrina* was designated by Keen (1969) as *Mactra plicataria* Linnaeus, and it must be considered an objective synonym of *Mactrinula*, published in January 1853 (Dall, 1915; Keen, 1969).

Finally the types of *Trinitasia iheringi* (Dall, 1897) and its synonym *Mulinia kempfi* Cauquoin, 1969, are illustrated (Figs. 56–59). This species was historically included in the genus *Mactrella*; however, its shell morphology suggests better placement in *Trinitasia*. A complete taxonomic and morphological review of *T. iheringi* was given by Signorelli & Pastorino (2012b).

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SPAWN AND REPRODUCTION OF THE GASTROPOD *TROCHITA PILEUS* (LAMARCK, 1822) FROM THE SOUTHWESTERN ATLANTIC OCEAN

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ABSTRACT

Specimens and eggs masses of the gastropod *Trochita pileus* were collected during a research cruise by bottom trawling at depths between 82 m and 120 m. Adult *T. pileus* were found attached to such hard substrata as the shells of scallops and oysters. The egg mass of *T. pileus* is composed of 7–8 transparent, triangular-shaped egg capsules fixed to the substratum with stalks. Four egg masses collected from brooding females, with a total number of 241 embryos, were examined. *Trochita pileus* is a protandrous hermaphrodite, and histological studies of the male, transitional, and female gonads were performed. The testicular portion of the gonad in both male and transitional individuals and the ovary of the female were studied by histological techniques. Spermatogenesis was observed for males and transitional individuals.

Key words: Egg capsules, protandry, Calyptraeidae, reproduction, Caenogastropoda.

INTRODUCTION

All members of the caenogastropod family Calyptraeidae are believed to be protandrous hermaphrodites (Coe, 1938; Wyatt, 1960; Gallardo, 1976; Collin, 2000). Hoagland (1986) first published a review of the development and reproduction of 30 species and described the different types of development observed in the calyptraeids. This was followed with additional information by Collin (2003). Five modes of development are present in the family. Either small or large eggs can develop directly into juveniles; additionally, small eggs can develop into small planktotrophic larvae, and large eggs can develop into pediveligers that swim before settling. Or pediveligers can develop from small eggs. Planktotrophic and direct development are the two most common modes of development in the calyptraeids; the presence of nurse eggs is common for this group, as is lecithotrophic development with a short-lived pediveliger stage (Gallardo, 1976; Collin, 2003).

Trochita pileus (Lamarck, 1822) is a common species living subtidally on hard substrata with a conical shell and a reported maximum diameter of 30 mm. Radial ribs are normally absent, but when present are narrow, and the septum margin is sigmoid (Pastorino & Urteaga, 2012). It occurs in Argentina from Buenos Aires (38°S) to the Patagonian coast (54°S) (Pastorino

& Urteaga, 2012). *Trochita pileus* is not an exploited resource, but is extremely vulnerable, because it is part of the bycatch with the *Zygochlamys patagonica* (King, 1832) fishery. *Zygochlamys patagonica* shells constitute hard substrata available for the settlement of sessile organisms, which contributes to increased benthic biodiversity in the continental soft-bottom areas where scallop beds are located (Schejter & Bremec, 2007).

We note herein a description of the spawn of *Trochita pileus*, including the gonad morphology at hatching stage of a population from Buenos Aires coast, Argentina, on the southwestern Atlantic Ocean. Other aspects of the biology of *T. pileus* are also described, including the histological characterization of the gonad portions of the female, the transitional stage, and the male of *T. pileus*.

MATERIALS AND METHODS

Adults specimens and egg masses of *T. pileus* were collected off Buenos Aires Province (37°33'S and 55°57'W) during a research cruise aboard of the Argentine B/I "Puerto Deseado". Samples were collected by bottom trawling using a research dredge (0.8 m mouth opening, 10 mm mesh size at the cod-end), at depths between 82 m and 120 m during June and July 2010. The bottom water temperature

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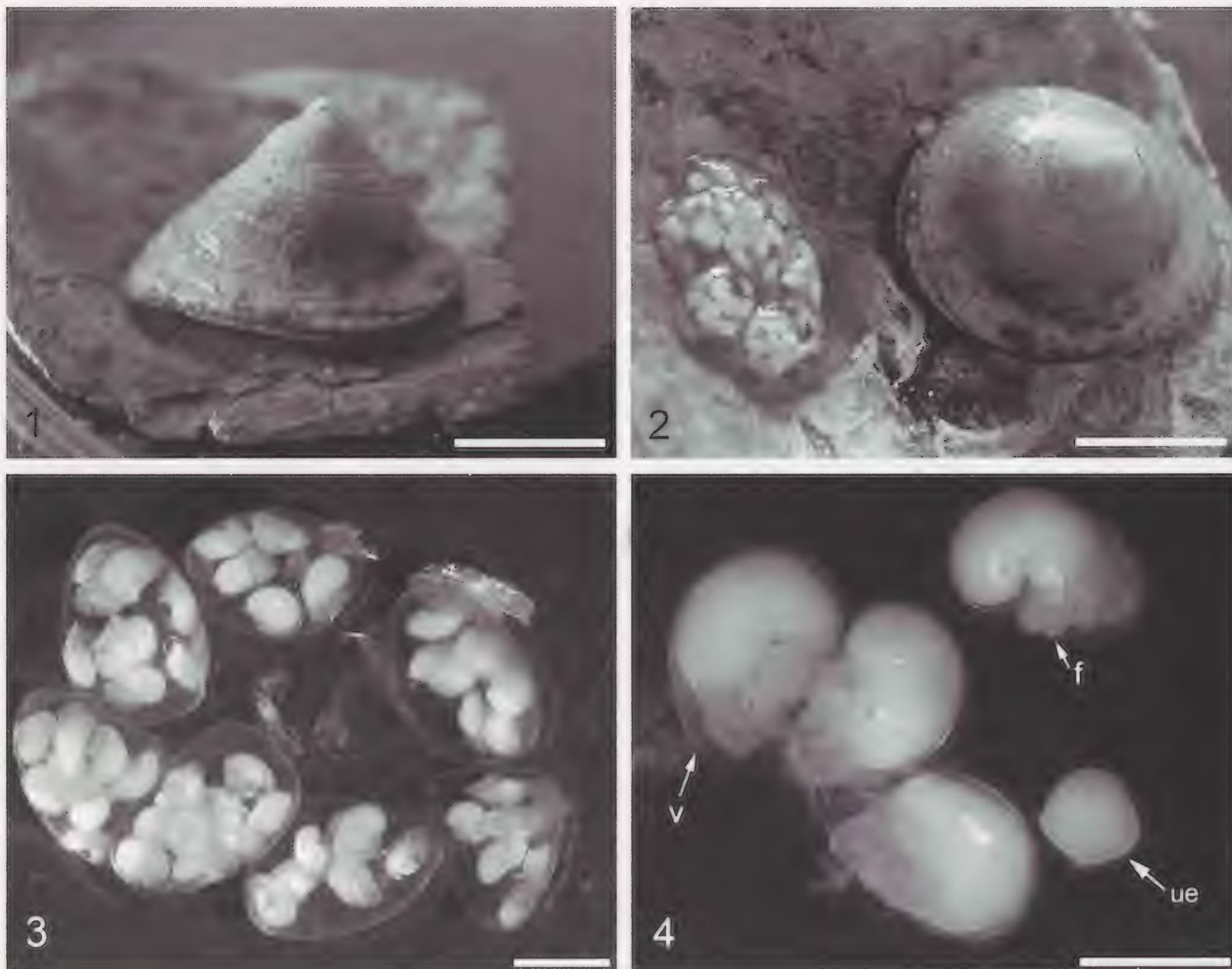
was 10.96°C, and conductivity was 33.48‰. Both were measured with a CTD Sea-Bird Electronics 911 Plus.

Trochita pileus were found attached to hard substrata, such as shells of scallops and oysters (Fig. 1). Immediately after collection, nine individuals were fixed in 5% formalin-seawater solution for 24 h, and stored in 70% ethanol. In the laboratory, *T. pileus* individuals were removed from the substrata and measured with a digital caliper at a precision of 0.01 mm. Nine individuals and four eggs masses were collected. For each capsule, the height (excluding the stalk), and the maximum width (apical region) were measured with a stereomicroscope. The egg masses are brooded under the shell, with the neck above the capsules and the substratum and propodium below them (Fig. 2).

The length of the eggs and the embryos were measured for each brood. The embryos in the broods were counted and photographed using a Zeiss Stemi 2000 C stereomicroscope.

For light microscopy, gonadal tissue was embedded in HistoResin using standard procedures. Serial sections (4 µm thick) were stained with a modified haematoxylin-eosin. Cell structures were determined by viewing and photographing tissue sections using a Zeiss Axiostar light microscope.

The reproductive state of individuals was characterized as immature when neither follicles nor sex cells were observed, as male when spermatozoa were observed, as a transitional individual when both an ovary and testis were found, and as female when only an ovary with gametes was observed.



FIGS. 1–4. Adults and egg masses of *Trochita pileus*. FIG. 1: *T. pileus* specimen attached to an oyster; FIG. 2: Egg mass and brooder adult; FIG. 3: Egg mass, composed by 7 ovicapsules; FIG. 4: Embryos at the early pediveliger stage; note presence of the velum (v) and incipient foot (f). unsegmented egg (ue). Scale bars: Figs. 1, 2 = 10 mm; Fig. 3 = 1 mm; Fig. 4 = 2 mm.

RESULTS

Specimens were attached to a hard substratum (Fig. 1). Egg capsules are brooded under the body of females (Fig. 2). The diameter of females ranged from 15.99–20.68 mm ($X = 19.38$ mm, $n = 5$, $SD = 1.93$). The three transitional individuals measured 7.11, 7.42 and 8.19 mm ($X = 7.57$ mm, $n = 3$, $SD = 0.55$) in diameter, and have a vestigial penis. The male diameter was 6.2 mm and exhibited a well-developed penis. No immature individuals were found in this range of sizes. We examined four egg masses for a total number of 241 embryos. Each egg mass was composed of 7–8 egg capsules, and 30 capsules were analyzed. *Trochita pileus* has transparent, triangular-shaped egg capsules with an attachment stalk (Fig. 3). For the egg capsules, the average height was 3.31 mm ($n = 28$, $SD = 0.55$), and the average maximum width was 3.98 mm ($n = 28$, $SD = 0.60$). The average stalk length was 3.14 mm. Each capsule contained 5–14 embryos showing a velum and, in most cases, an incipient foot. These characters allowed us to classify these embryos as being in the early pediveliger stage. In some cases, we found uncleaved eggs. Development was synchronous within and among capsules. Shell length at the embryo stage was 0.72–1.6 mm ($X = 1.26$ mm, $n = 241$, $SD = 0.14$) (Fig. 4).

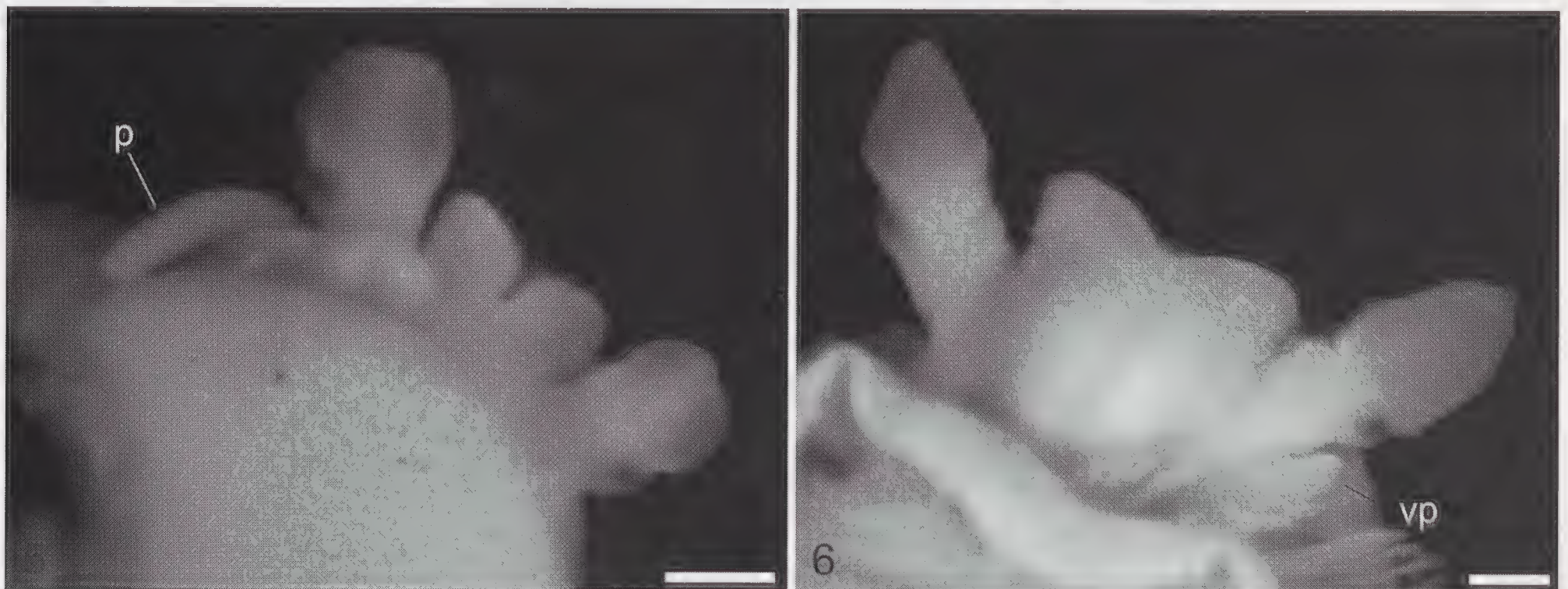
Transitional individuals still have a well-developed penis (Fig. 5). Females have a vestigial penis just posterior to right cephalic tentacle (Fig. 6). Histological studies of the gonads of

males, transitional individuals, and females were performed.

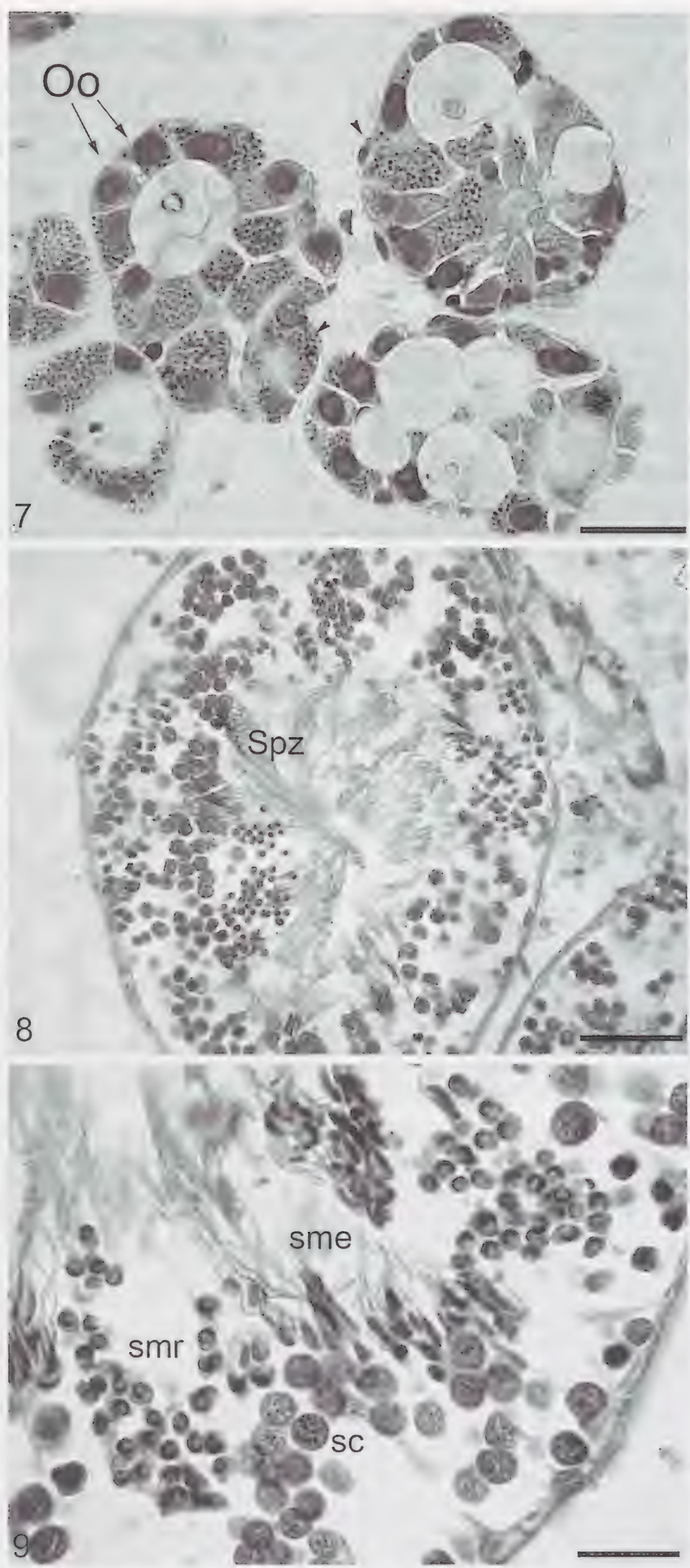
In individuals larger than 15.9 mm, we found only ovaries (Fig. 7). The histological analysis suggested that spawning in *T. pileus* is not complete, because we found oocytes remaining in the gonad after spawning (Fig. 7). For specimens less than 7 mm, we found only a testis, and we found a testis and an ovary in the individuals of the transitional phase, between 7 and 8 mm. The testis consisted of numerous spermatogenic tubules separated from each other by connective tissue (Fig. 8). Within each spermatogenic tubule, the spermatogenic cells were situated towards the interior side of the basal membrane. Spermatogenic cells, spermatogonia, spermatocytes, ring-shaped, elongate spermatids were generally clustered in small groups in the same maturation phase and are distributed throughout the tubule. Mature spermatozoa generally filled the lumen of the tubule (Fig. 8). In transitional individuals, the spermatogenesis occurs similarly to male individuals; details show the different stages of spermatogenic cells (Fig. 9).

DISCUSSION

Protandric hermaphroditism is frequently found in gastropods (Coe, 1938). About 40% of mollusc genera are either simultaneous or sequential hermaphrodites (Heller, 1993). Calyptraeids have a reproduction cycle with



FIGS. 5, 6. Anterior view of *T. pileus*. FIG. 5: Ventral view of *T. pileus* male, detail of the penis (p) in male (1.3 mm); FIG. 6: Dorsal view of the female, detail of the vestigial penis (vp) (0.5 mm) in a female (19 mm SL). Scale bars: Fig. 5 = 1 mm; Fig. 6 = 0.5 mm.



FIGS. 7–9. Light micrographs of histological sections of *T. pileus*. FIG. 7: Ovary; note the presence of oocytes (oo) and the vitellogenic oocytes (arrowhead). Scale bar = 0.5 mm; FIG. 8: Transversal section of the spermatogenic tubule in transitional individual, with different stages of spermatogenesis. Scale bar = 50 μ m; FIG. 9: Detail of testis of a transitional individual showing sperm (spz) at the lumen, spermatocytes (sc), and spermatids in the ring stage (smr) and elongated stage (sme). Scale bar = 20 μ m.

a sequence of sexual phases (Heller, 1993; Collin, 2000; Chaparro et al., 2001; Cledón & Penchaszadeh, 2001; Simone, 2002; Chaparro et al., 2005; Collin et al., 2005). Wyatt (1960) described five stages or sexual phases for *Calyptraea chinensis* (Linnaeus, 1758): immature male, functional male, associated male (in species where individuals do not form chains and males associate with females only for copulation), immature females, and females. During the transition period, morphological changes from the male phase to the female phase were observed. The penis is absorbed and the reproductive system is reorganized; transitional individuals have a penis in retraction (Coe, 1938). Usually, there are several phases: undifferentiated juveniles, males, transitional individuals and females (Coe, 1938; Wyatt, 1960; Heller, 1993; Brown & Olivares, 1996; Gallardo, 1996; Collin, 2000, 2003). In the present study, male, transitional individuals and females were analyzed. The individuals at the male phase and the transitional phase have a well-developed penis. The female phase showed a vestigial penis. The successive stages in the development of the reproductive system in the two sexual phases are correlated with the development of secondary sexual characters. Males have a penis, and transitional individuals and females present a vestigial penis just posterior to right cephalic tentacle.

Sometimes the transition from the male to the female phase takes place rapidly, but often there is a prolonged period of apparent inactivity, during which the gonadal follicles contain few oogonia. The duration of the transitional period is related to the isolation degree for individuals (Coe, 1938).

Egg Capsules and Developmental Pattern

Caenogastropods show a considerable morphological variation in spawn, ranging from gelatinous masses attached to hard substrata at the bottom to pelagic capsules. Capsules may be complex, with delicate to tough walls, and their shape and size can vary within families and genera (Thorson, 1946; Fretter & Graham, 1962). There are many kinds of parental care in caenogastropods. Adults can defend their spawn from predators actively, whereas others brood capsules in the mantle cavity, oviduct, foot or even a special chamber in the head (Ponder et al., 2007). All calyptraeids show parental protection during brooding; it involves the physical care of an egg mass or cluster of

capsules, which are attached to substratum under the female (Collin, 2003). Calyptraeids are characterized by the production of stalked egg capsules with a thin membrane (Gallardo, 1979; Hoagland, 1986; Chaparro et al., 1999; Cledón & Penchaszadeh, 2001). *Trochita pileus* has transparent, triangular-shaped egg capsules with a fixation stalk, and exhibits parental care.

The fixation stalk length is related to the position of the incubated egg mass. Brooding over the head requires a longer fixation stalk or peduncles compared to brooding between the head and the propodium (Hoagland, 1986). Egg capsule morphology is not a taxonomically useful character according to Hoagland (1977); however, for species in which this character has been described, differences in shape or size could have taxonomic value (Gallardo, 1977, 1979).

Calyptraeids present various reproductive strategies; some species have nurse eggs, whereas other do not. Also there is considerable variation in the morphology and consumption of nurse eggs (Collin, 2003).

We did not find enough evidence to affirm there are nurse eggs in *T. pileus*. We found only two unsegmented eggs from a total 241 embryos in the same development stage. Collin (2000) also described synchronous development for *Crepidula adunca* (G. B. Sowerby I, 1825) and *Crepidula lingulata* (Gould, 1846).

There are a few descriptions about the gonadal histology of these species (Chaparro et al., 1998). The testis is composed of numerous spermatogenic tubules where the spermatogenesis, which is responsible for the production of sperm, occurs. Our observations show that spermatogenesis occurs in male and transitional individuals.

Further studies are necessary to elucidate the spermatogenesis and sperm ultrastructure of these species. Studies employing transmission electron microscopy would expound upon these reproductive aspects.

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THRESHOLD TEMPERATURES AND DEGREE-DAY ESTIMATES
FOR EMBRYONIC DEVELOPMENT OF THE INVASIVE APPLE SNAIL
POMACEA CANALICULATA (CAENOGASTROPODA: AMPULLARIIDAE)

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ABSTRACT

Pomacea canaliculata is a freshwater snail native to subtropical-temperate South America that has invaded several countries around the world. Temperature is probably one of the main limitations to the expansion of this and other apple snails to higher latitudes in invaded regions. Egg masses are aerial, and the duration of embryonic development varies greatly with air temperature. We compared different methods for determining the lower temperature thresholds and the cumulative degree-days (DD) required for the completion of the embryonic development of *P. canaliculata*. The lower temperature threshold was estimated with four methods: the least standard deviation from the mean of degree-days, the least standard deviation from the mean of days, the coefficient of variation in days and the linear regression coefficient method. The cumulative degree-days were estimated using hourly records and daily averages (calculated according to the single triangle and the single sine methods) of air temperature. The lower temperature thresholds ranged between 15.8°C and 18.3°C and the cumulative DD between 88.8°C.d and 133.8°C.d. The estimations obtained with the single triangle and the single sine methods were exactly the same. The values obtained with the method of the least standard deviation in degree-days and the corresponding cumulative DD were the poorest estimations. The estimates obtained with daily mean temperatures were close to those obtained with hourly records, indicating that recording only maximum and minimum temperatures should be sufficient. The use of degree-day models for egg development in *Pomacea* will serve to increase the effectiveness and efficacy of control measures targeted to egg masses through a better timing in their application, especially in localities with highly variable temperatures.

Key words: eggs, hatching, lower threshold, cumulative degree-days, developmental rate.

INTRODUCTION

Pomacea canaliculata (Lamarck 1822) is a freshwater snail native to subtropical-temperate South America naturally distributed from Paraguay and southern Brazil southwards to southern Buenos Aires Province, Argentina (Martín et al., 2001; Hayes et al., 2008). This species and a few congeners are serious pests in several countries around the world where they have invaded different types of freshwater systems, such as paddy fields and natural wetlands (Cowie, 2002; Rawlings et al., 2007; Hayes et al., 2008; Lv et al., 2012), prompting the listing of *P. canaliculata* among the 100 worst invaders worldwide (Lowe et al., 2000). Temperature is probably one of the main limitations to the

expansion of apple snails to higher latitudes in invaded regions (Baker, 1998; EFSA, 2012).

Many aspects of the biology of *P. canaliculata* depend on temperature, such as growth rate, reproduction, lung ventilation frequency, feeding rate and activity (Estebenet & Martín, 2002; Seuffert et al., 2010). In temperate climates, *P. canaliculata* reproduces more than once during its lifespan, whereas under tropical conditions it behaves as semelparous, showing only one reproductive period (Estebenet & Cazzaniga, 1992). Females deposit aerial, pink calcareous eggs, arranged in clusters of 30 to 300 eggs (Albrecht et al., 1996; Tamburi & Martín, 2011). Embryonic development occurs within the calcareous egg capsules and hatching usually occurs two weeks after oviposition, but this

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period varies greatly (Estebenet & Cazzaniga, 1992; Pizani et al., 2005; Liu et al., 2012), most of this variation being probably caused by the inverse relationship of air temperature and developmental rate.

The developmental rate of ectotherms is dependent on temperature, so that the cooler the temperature, the slower is the rate of growth and development. As temperature increases above a specific minimal threshold, development time progressively decreases, until the temperature becomes high enough to affect growth and development negatively. The lower developmental threshold for a species is the temperature at and below which development stops. The upper developmental threshold is less well defined, but it is commonly considered as the temperature at and above which developmental rate does not increase (Zalom et al., 1983; Snyder et al., 1999; Ruml et al., 2010).

As temperature increases, the duration of a specific physiological process decreases, but the cumulative heat required remained approximately constant (Zalom et al., 1983; Snyder et al., 1999; Lv et al., 2011). Temperature could be constant or fluctuating, but the combination of temperature (above the lower threshold) and time will be the same. This physiological time for development is measured in cumulative degree-days. A bigger difference between the temperature and the lower threshold implies an earlier accumulation of the required degree-days and hence a faster developmental rate.

Zhou et al. (2003) developed a degree-day model for *P. canaliculata* and estimated a threshold temperature for egg development of 14.0321°C. However, this parameter was obtained from populations living in an invaded area (China) and of which the identity is uncertain, because at least another *Pomacea* species coexist with *P. canaliculata* in mainland China (Hayes et al., 2008; Lv et al., 2012), and it is difficult to distinguish them externally (Cowie et al., 2006). Given that the threshold temperature for development may vary considerably as consequence of intrinsic features of the snails and factors related to specific environmental conditions, it is important to investigate the thermal requirements of *P. canaliculata* from data obtained from native individuals in its natural realm. Moreover, studies on the thermal requirements for embryonic development of snails with aerial eggs are very scarce (Liu et al., 1991; Zhou et al., 2001, 2003) and hence this work will contribute to the general knowledge of the thermal biology of gastropods.

Most studies on the degree-days required to hatching in freshwater gastropods have been developed on the basis of the variation of development duration of eggs incubated at different constant temperatures (e.g., Zhou et al., 2001, 2003; Hong et al., 2004). Here we took a different approach, computing thresholds and cumulative degree-days to hatching for *P. canaliculata* on the basis of hourly or daily records of fluctuating temperatures, an approach that has been successfully applied in other ectotherms (e.g., Yang et al., 1995; Snyder et al., 1999; Ruml et al., 2010). Although using hourly data provides a higher accuracy in the calculation of degree-days, daily maximum and minimum temperature data can also be used and they are often the only available records.

The aims of this work are to compare different methods for determining lower temperature thresholds, selecting those that are best supported by the data recorded for numerous egg masses, and to estimate the degree-days required for the completion of the embryonic development of *P. canaliculata*. These results will expand the knowledge of the thermal biology of this invasive species and would be useful for the development of predictive models of distribution both in native and invaded areas and to increase the efficiency of control measures directed towards the egg masses, the most conspicuous stage of the life cycle.

MATERIALS AND METHODS

Egg Masses and Temperature Records

Thirty males and 30 females of *P. canaliculata* were hand-collected in November 2009 at Guaminí stream (37°10'44"S, 62°25'59"W, Encadenadas del Oeste basin, Buenos Aires Province, Argentina). In the laboratory (located 170 km to the SSE from the collection site), 30 couples were formed and put in 3 L aquaria with CaCO₃ saturated tap water and fed with lettuce. From this pool of couples, 24 females that have laid at least one egg mass during the first week were selected for further record of their ovipositions.

From November 25, 2009, to April 26, 2010, the females were reared under the same conditions in a room without control of photoperiod or temperature. The experiment was ended after four weeks had passed without any oviposition. Half of the females received a full ration of lettuce corresponding to an *ad libitum* ingestion

rate (estimated from the equation provided by Tamburi & Martín, 2009) and the others received 50% of that ration. Water was changed and the aquaria were cleaned weekly. Every four weeks, a male was put in the aquarium during one day to maintain sexual activity and to allow replenishment of sperm reserves. The presence of egg masses fastened to the aquarium wall was checked every day in the morning, because egg laying is mostly a nocturnal activity (Albrecht et al., 1996; Estebenet & Martín, 2002). Once an egg mass was detected, the female together with the water, lettuce remains and feces were changed to a new aquarium. The egg mass was incubated in the original aquarium (with 1 cm of tap water in the bottom) in the same room as females; room's air temperature (T_h , °C) was recorded hourly with a Hobo® data-logger. The egg masses were checked daily to record the appearance of hatchlings in the aquarium. The counting of hatchlings was ended after one week without any hatching and the remaining eggs were counted to estimate egg mass viability. The duration of development (d, days) was calculated as the number of days elapsed between egg laying and the date of first hatching.

Determination of Threshold Temperature

The lower temperature threshold (T_l) for embryonic development was estimated with four different methods: (1) the least standard deviation from the mean of degree-days (SD-DD) proposed by Magoon & Culpepper (1932) and Stier (1939), (2) the least standard deviation from the mean observed number of days (SD-day) of Arnold (1959), (3) the coefficient of variation in days (CV-day) of Nuttonson (1958) and (4) the linear regression coefficient method (REG) from Hoover (1955). The different threshold temperatures were estimated using the equations developed by Yang et al. (1995) based on these four methods. To confirm that the three first equations perform properly, an iterative process with different candidate threshold temperatures was performed, as suggested by Snyder et al. (1999). The value that produced the least standard deviation in the mean of DD (1), in the mean of days (2) and the least coefficient of variation in days (3) was compared to the values obtained with the equations proposed by Yang et al. (1995).

For all methods, two sets of temperature data were used (making a total of eight estimations of the lower threshold): the detailed hourly

temperature data and a daily mean calculated averaging the records of maximum and minimum temperatures of each day.

Estimation of Degree-days Requirement

The degree-days (DD, °C.d) for the embryonic development of *P. canaliculata* were calculated using the hourly records of temperature by computing the degree-hours and summing over the 24-h. Degree-hours were calculated as the difference between the recorded hourly mean temperature (T_h) and the lower threshold (T_l):

$$DD = \sum (T_h - T_l)$$

The cumulative DD were estimated by summing the daily DD over the specific period of development (d) of each egg mass. We also estimated the cumulative DD using a mean value of temperature (T_m) obtained from daily highest (T_x) and lowest (T_y) records. This mean temperature was calculated according to the single triangle and the single sine methods described by Zalom et al. (1983). For all estimations, DD was set to zero when $T_l > T_h$ or T_m since it was reported that it generally gave better results than the approach in which DD retained a negative value (Ruml et al., 2010). The three methods were applied in combination with the eight estimations of the lower temperature threshold, giving a total of 24 estimations of cumulative DD.

In this work, we considered the introduction of an upper threshold to be unnecessary since, based on the evidence reported in previous works (e.g., Cowie, 2002; Seuffert et al., 2010), it may be inferred that the highest temperature to which the egg masses were exposed here (31.93°C) almost certainly does not preclude development.

RESULTS

A total of 123 egg masses were laid by the 24 females during the whole period of oviposition (late spring to early autumn). The mean viability of egg masses (number of live hatchlings / total number of eggs) was 35.5% and fluctuated between 0 and 99%. For the estimations of thresholds and degree-days, we decided to use the data obtained from egg masses with viabilities greater than 10%, leaving a total of 88 egg masses. The time required for embryonic development (d) varied from a minimum of only 7 days to a maximum of 30 days (mean $\pm$ SD = 14.67 ± 3.44 days).

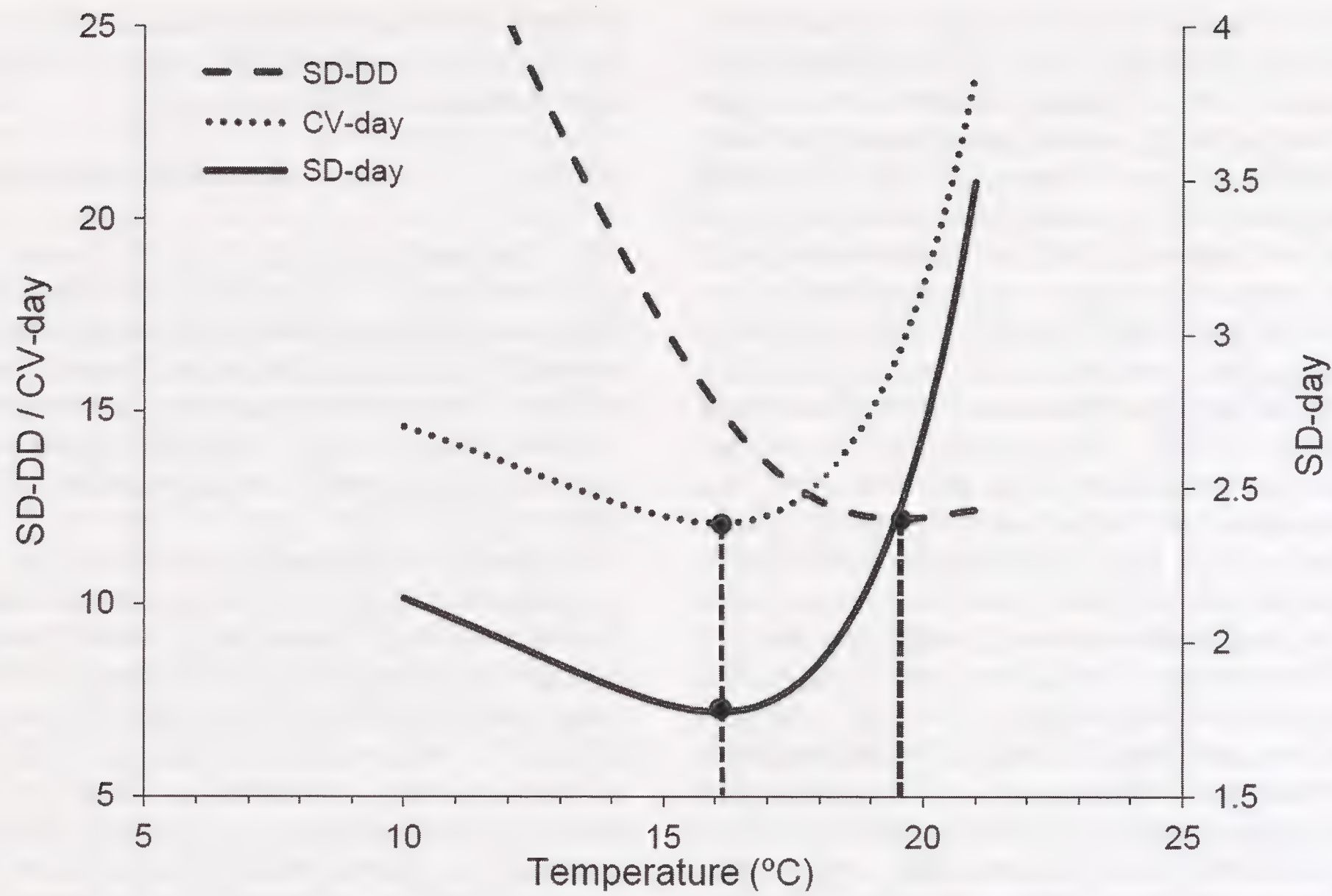


FIG. 1. Standard deviation in degree-days (SD-DD), standard deviation in the observed number of days (SD-day) and coefficient of variation in days (CV-day) for the embryonic development of *Pomacea canaliculata* (estimated with the hourly records of temperature) vs. temperature. The dotted vertical lines show the minimum value for the three methods corresponding to the lower temperature thresholds (19.6°C, 16.1°C and 16.1°C, respectively).

TABLE 1. Threshold temperatures and degree-days (DD) for the embryonic development of *Pomacea canaliculata*. Two data sets of temperature (T°) were used: hourly records and a daily mean of the maximum and minimum records. Threshold temperatures (°C) were estimated by different methods: the least standard deviation in DD (SD-DD), the least standard deviation in days (SD-day), the coefficient of variation in days (CV-day) and the linear regression coefficient method (REG); values in parentheses are the thresholds obtained by iteration. The cumulative DD (mean ± SD; °C.d) were estimated using the hourly temperature records (hourly) and the highest and lowest records (triangle and sine methods).

T° Data sets	Method	Threshold	Cumulative DD		
			hourly	triangle	sine
Hourly records	SD-DD	18.2 (19.6)	90.3 ± 12.6	94.8 ± 12.7	94.8 ± 12.6
	SD-day	15.8 (16.1)	125.0 ± 15.6	132.2 ± 15.1	132.2 ± 15.1
	CV-day	15.9 (16.1)	120.7 ± 15.0	130.7 ± 15.0	130.7 ± 15.0
	REG	16.0	122.1 ± 15.2	129.1 ± 14.8	129.1 ± 14.8
Daily mean	SD-DD	18.3 (18.6)	88.8 ± 12.5	93.2 ± 12.6	93.2 ± 12.6
	SD-day	15.7 (15.8)	126.5 ± 15.7	133.8 ± 15.3	133.8 ± 15.3
	CV-day	16.1 (15.8)	120.7 ± 15.0	127.6 ± 14.6	127.6 ± 14.6
	REG	16.2	119.2 ± 14.8	126.0 ± 14.5	126.0 ± 14.5

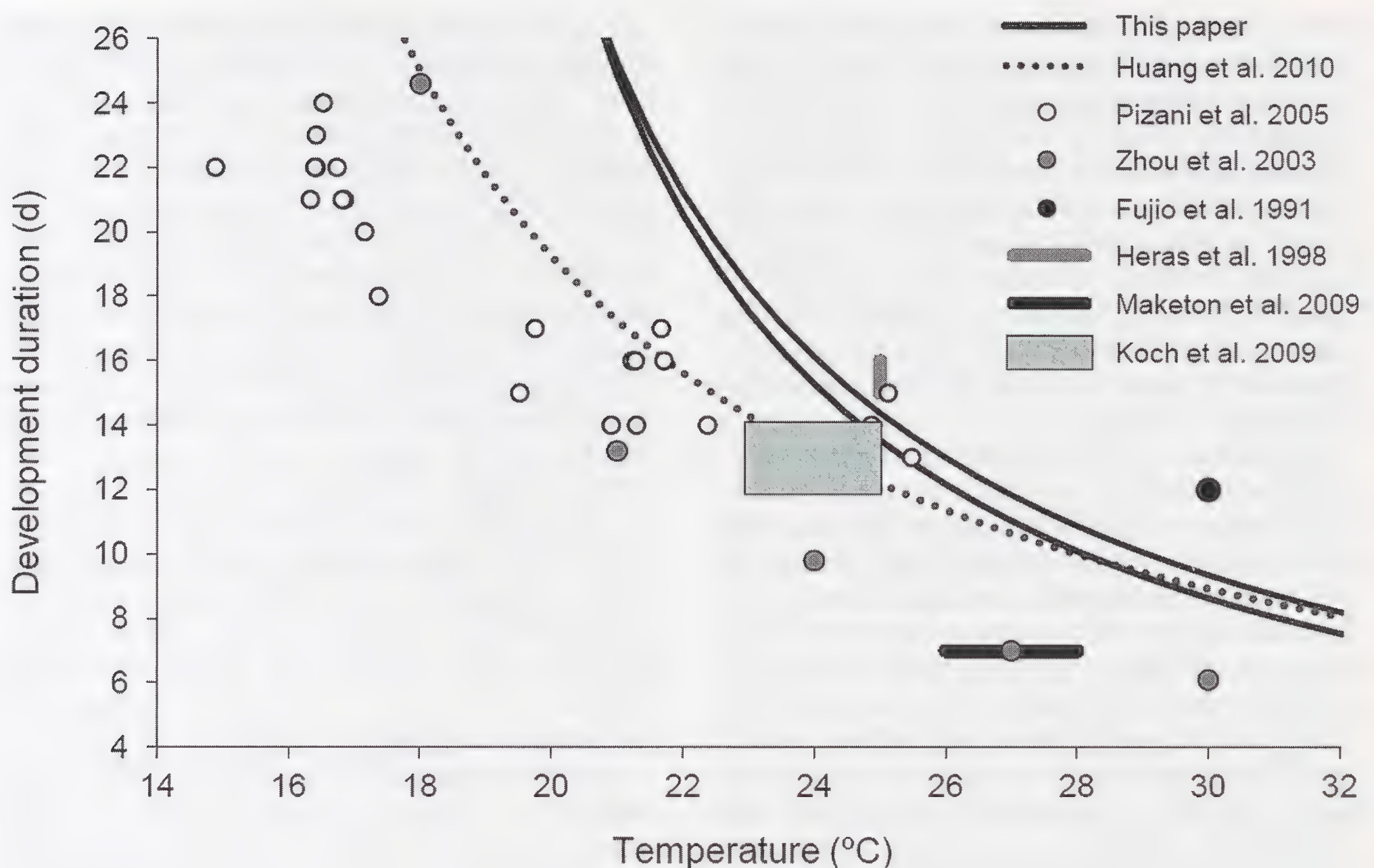


FIG. 2. Duration of embryonic development of *Pomacea canaliculata* at different temperatures (continuous lines) calculated with the extreme estimations obtained in this paper (15.7°C and 16.2°C for the lower temperature threshold and 133.8°C.d and 119.2°C.d for the cumulative degree days) and durations reported by different authors (bars and rectangles indicate ranges of temperatures and/or durations, empty circles represent records for single egg masses and full circles averages for several egg masses; the dotted line was calculated on the basis of the linear regression model from Huang et al., 2010).

The two feeding regimes used here were scheduled as part of another study and will be analyzed elsewhere. However, it is worthy to note that a separate estimation of the mean DD (using the hourly records of temperature and the threshold of 15.8°C obtained with the SD-day method) revealed no significant differences between both treatments (t-test, $p = 0.906$).

Determination of Threshold Temperature

The lower temperature thresholds for the embryonic development of *P. canaliculata* estimated with the equations of Yang et al. (1995) for the four methods fluctuated between 15.8°C and 18.3°C (Table 1). The values obtained by iteration (Fig. 1) were very similar to those obtained with these formulae for SD-day and CV-day methods (differences not bigger than 0.3°C) while for the SD-DD method the differences were larger (up to 1.4°C) between both threshold estimations.

Estimation of Degree-days Requirement

The mean cumulative DD estimations ranged between 88.8°C.d and 133.8°C.d (Table 1). The estimations obtained with the single triangle and the single sine methods were exactly the same. The values estimated from the hourly temperature records were 4.72% to 7.65% lower than both cumulative DD obtained from the daily mean of maximum and minimum records.

DISCUSSION

The threshold values obtained with the SD-DD method were higher (between 11.0% and 14.2%) than the other estimations and differed more from the corresponding values obtained by iteration. Besides, a threshold of 18.2°C or 18.3°C seems too high as compared to independent estimations for *Pomacea* species (15.4°C, Liu et al., 1991; 14.03°C, Zhou

et al., 2003). We therefore concluded that the values obtained by this method (with both sets of temperature data) and the corresponding cumulative DD (see below) are the poorest estimations for the embryonic development of *P. canaliculata* and hence will not be considered hereafter. The same situation was reported by other authors: in some cases, the SD-DD method generated negative values (Črepinšek et al., 2006; Ruml et al., 2010) and in others produced poor predictions (Snyder et al., 1999; Yang et al., 1995).

Ruml et al. (2010) showed that the results obtained with the CV and SD in days' formulae from Yang et al. (1995) are almost identical. With our data, the results obtained with those two methods were also very similar when using the formulae and, moreover, were exactly the same when we calculated them by iteration. As such, we infer that any of these two methods would be adequate and will produce very similar results.

On the other hand, the cumulative degree-days estimated using only the highest and lowest daily temperatures were exactly the same when using the single triangle and single sine methods. This situation occurs when temperature records fall within the range comprised by the lower and upper thresholds (Zalom et al., 1983). In our daily temperatures, only four records were below the lower threshold, being recorded at the end of the oviposition period (late April) and were considered for only one egg mass calculation. However, it has been reported that both the single triangle and single sine methods are easily calculated and are good estimators (Zalom et al., 1983). Therefore, even with a wider range of temperatures either of these methods would provide acceptable estimates when only daily temperature measures are available.

The estimates obtained using daily mean temperature values were close to those obtained using hourly records (either for the estimation of the lower thresholds or the calculation of cumulative degree-days). This indicates that they provide the best measure when hourly records are available, but when designing a new experiment, recording only maximum and minimum temperatures should be sufficient (see below).

The simplest way to calculate the cumulative DD is to use 0°C as threshold but this approach seems to be appropriate only for organisms with very low threshold temperatures, as is the case of several plant species (e.g., Snyder et al., 1999; Črepinšek et al., 2006; Ruml et

al., 2010) or the eggs of Siberian freshwater limpets (Shirokaya & Röpstorf, 2003). However, this 0°C-threshold is not adequate for a subtropical-temperate snail species whose feeding, crawling and aerial respiration cease between 10°C and 15°C (Seuffert et al., 2010). The use of 0°C as threshold could result in a significant overestimation of cumulative degree-days requirement for this species, just as the high values obtained with the SD-DD method (18.2–18.3°C) produced very low values of cumulative degree-days relative to the other methods (Table 1).

The range of temperature thresholds for embryonic development reported here (15.7–16.2°C) is just two degrees higher than those reported for nonindigenous populations of this species (14.03°C, Zhou et al., 2003; 14.2°C, Huang et al., 2010), and a half degree higher than the threshold reported for an uncertain *Pomacea* species identified as *Ampullaria gigas* (15.4°C, Liu et al., 1991). The reported cumulative degree-days for *Pomacea* are more variable than those of temperature thresholds and our values (119.2–133.8°C.d) lay well within the range reported (94.87°C and 161.3°C.d; Liu et al., 1991; Zhou et al., 2003; Huang et al., 2010). Our snails came from one the southernmost natural populations of *P. canaliculata*, where low temperatures set a limit to different activities during half of the year (Seuffert et al., 2010) and where a lower threshold would prevent excessively extended development periods. The ecological significance of these differences is difficult to assess. Part of the variation in these parameters may be explained by the different methodology employed as estimates from Chinese populations were obtained from egg masses incubated at different constant temperatures. Furthermore, differences between apple snail populations in the native range and those elsewhere may be due to acclimation, genetic drift or hybridization in the invaded range, different geographic sources of origin in the natural range, or simply species misidentification (e.g., Lv et al., 2012; Hayes et al., 2008).

The duration of embryonic development of *P. canaliculata* at different temperatures predicted by our degree-day models was compared to the durations reported by other authors (Fig. 2). The durations reported for egg masses under constant temperatures in their natural range (Buenos Aires Province, Argentina; Heras et al., 1998; Koch et al., 2009) only differed from our predictions in two days at most. In contrast,

estimates obtained under constant temperatures with populations in the invaded range of Southeastern Asia were generally lower than predicted, from 2 to 13 days shorter at 32°C and 21°C, respectively (Maketon et al., 2009; Zhou et al., 2003). The values predicted by the model proposed by Huang et al. (2010) were the same as ours at high temperature but became increasingly smaller as temperature decreases, reaching a difference of 12 days at 20°C. Again, the information available on thermal ecology of *Pomacea* egg development is not complete enough to determine if the observed differences are methodological, taxonomic or genetic in origin. For instance, the duration of 12 d at 30°C reported by Fujio et al. (1991) is clearly the oddest and was obtained in Japan for a laboratory strain in which low hatchability was associated with inbreeding depression.

Pizani et al. (2005) published data on the mean temperature and duration of development for egg masses incubated under uncontrolled temperature in the same laboratory as those of the present study and deposited by snails from the same geographic area (Encadenadas del Oeste basin). The temperature required to hatch in a certain number of days was clearly lower than that predicted by our degree days models (Fig. 2), especially for the egg masses that developed at lower rates. For instance, at 16°C the eggs hatched in 22–24 days but the predictions of our models indicate that at that temperature hatching would take an infinite time. This is probably due to the use of just one overall mean temperature (estimated over the entire incubation period for each egg mass) as an indicator of the thermal environment. Actual temperatures that fell well below the mean value of 16°C did not further retard development in those egg masses, but those well above 16°C certainly increased development time. Therefore, when temperature actually falls frequently below the threshold, the use of overall mean temperatures during the development indicates a lower threshold that is clearly lower than the temperature at which development can be completed according to our estimates.

A variety of control measures have been employed or tested against invasive *Pomacea* species in rice, taro and other paddy field crops but the results are far from satisfactory (e.g., Wada, 2004; Ranamukhaarachchi & Wickramasinghe, 2006). Among the control measures,

spraying of egg masses with water (Wang et al., 2012) or low toxicity hatching inhibitors (Wu et al., 2005) and collection or destruction of eggs *in situ* (e.g., Adalla & Magsino, 2006; Yang et al., 2006) have been proposed as environmentally friendly options. The extended reproductive season and the short development duration in tropical and subtropical areas implicates that the application of these treatments needs to be numerous and frequent. Moreover, these procedures would be ineffective if a certain number of eggs hatch between consecutive treatments or inefficient if, to avoid this possibility, the treatments are applied more often than actually required. The use of degree-day models for egg development in *Pomacea* will serve to increase the effectiveness and efficacy of these control measures through a better timing in their application, especially in localities with highly variable temperatures. According to our results, a simple and economic min-max thermometer located in the field would be sufficient to achieve a reduction in costs of spraying or egg mass harvesting and thence its application would be suitable even with low levels of technology and in low profit areas.

The estimation of lower thresholds and cumulative degree-days for embryonic development on the basis of hourly or daily records of fluctuating temperatures provided results comparable to those already available for *Pomacea* spp. and computed on the basis of development duration at different constant temperatures (e.g., Liu et al., 1991; Zhou et al., 2003). Additionally, our general approach is more economical as it does not involve special equipment (temperature controlled rearing chambers), instead it only requires a max-min thermometer (or a thermograph or data-logger if available) and it can also be applied in the field. This would permit the development of specific degree-day models at a local scale even if apple snails are not correctly identified.

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ON THE PROCESSES SHAPING SMALL-SCALE POPULATION STRUCTURE IN *RADIX BALTHICA* (LINNAEUS 1758)

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ABSTRACT

The evolutionary processes generating population genetic structures may differ depending on the spatial scale. We examined the population structure of the freshwater snail *Radix balthica* on a local scale in Saanen Valley, Switzerland. We used a combination of a direct approach by simulating dispersal with parameters gained out of experiments in an artificial pond and indirect molecular genetic approaches by using mitochondrial (COI) and nuclear (microsatellite) marker at all sites harbouring *R. balthica* in this restricted area.

Contrary to the expectations from the direct methods, populations in homogeneous water bodies were found to be genetically heterogeneous, independently of geographic distance between sites. Instead, several selfing lineages coexisted with little local gene flow among them.

The population structure observed in the valley could thus not be explained by active dispersal alone. It was rather characterized by independent, probably bird-mediated, colonisation events from several sources, in combination with high metapopulation dynamics and a mixed mating system preserving this structure. This population structure has rarely been reported before, but might nevertheless be typical for passively dispersed, patchily distributed taxa (e.g., freshwater invertebrates).

Key words: Basommatophora, computer simulation, neighbourhood, selfing, microsatellite marker, adaptation.

INTRODUCTION

Disentangling the processes governing spatial genetic structure is essential to understand the ecological and evolutionary dynamics of a species. Spatial genetic structure is shaped by the interaction of demography, natural selection, mating system and dispersal-mediated gene flow. However, the relative importance of these factors may vary according to spatial scale. On a local scale, active dispersal may play a more important role than long-range dispersal and vice versa on a broader scale.

Furthermore, a mixed mating system can substantially affect the distribution of genetic variation within and among populations (Charlesworth, 2003). Predominately selfing (sub-) populations are strongly influenced by drift due to a very low effective population size and are ultimately subject to genetic impoverishment (Jarne & Städler, 1995).

A recent study (Pfenninger et al., 2011) on the range-wide population structure of *Radix balthica* (Lymnaeidae) revealed a population structure characterised by the absence of isolation-by-distance, together with rather isolated and genetically depauperate populations compared to the variation present in the entire range. This seemingly paradoxical pattern was explained by distance-independent, passive long-range colonisation and high population turn-over rates, low gene-flow and/or strong drift, enhanced by the mixed mating system of the species (Pfenninger et al., 2011). This pattern of population structure, probably typical for passively dispersed, patchily distributed taxa (e.g., freshwater invertebrates), is likely to impede local adaptation (Kawecki & Ebert, 2004).

Radix balthica occurs in permanent slow-flowing rivers and stagnant water bodies, without requiring a particular substratum or high water quality (Økland, 1990; Glöer & Meier-Brook,

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1998). The individual active dispersing capacity is restricted to the water body the snail lives in. The egg clutches are attached to structures, and there is no free larval stage allowing dispersal (Glöer & Meier-Brook, 1998). Still, *R. balthica* is widely distributed over northwestern Europe (Pfenninger et al., 2006), implying extensive passive transport, as suggested for many freshwater taxa (e.g., Frisch et al., 2007). Many gastropods have a mixed mating system with outcrossing and selfing both common (Jarne & Städler, 1995; Meunier et al., 2001), which is also true for *R. balthica* (Pfenninger et al., 2011).

To understand the regional and local population structure of *R. balthica* in relation to the range-wide structure, we used direct (dispersal studies) and indirect (molecular genetic) methods at all sites harbouring *R. balthica* in a restricted area. In particular, we asked the following questions:

What are the expectations for local (intra water body) population structure of *R. balthica* based on active dispersal, and how do they correspond to the structure as inferred from molecular markers?

Are the populations connected by gene-flow on a regional scale, that is, in an area of 56 km²?

How does the inferred structure relate to the large-scale population over the whole area of distribution structure?

METHODS

Direct Estimation of Population Structure

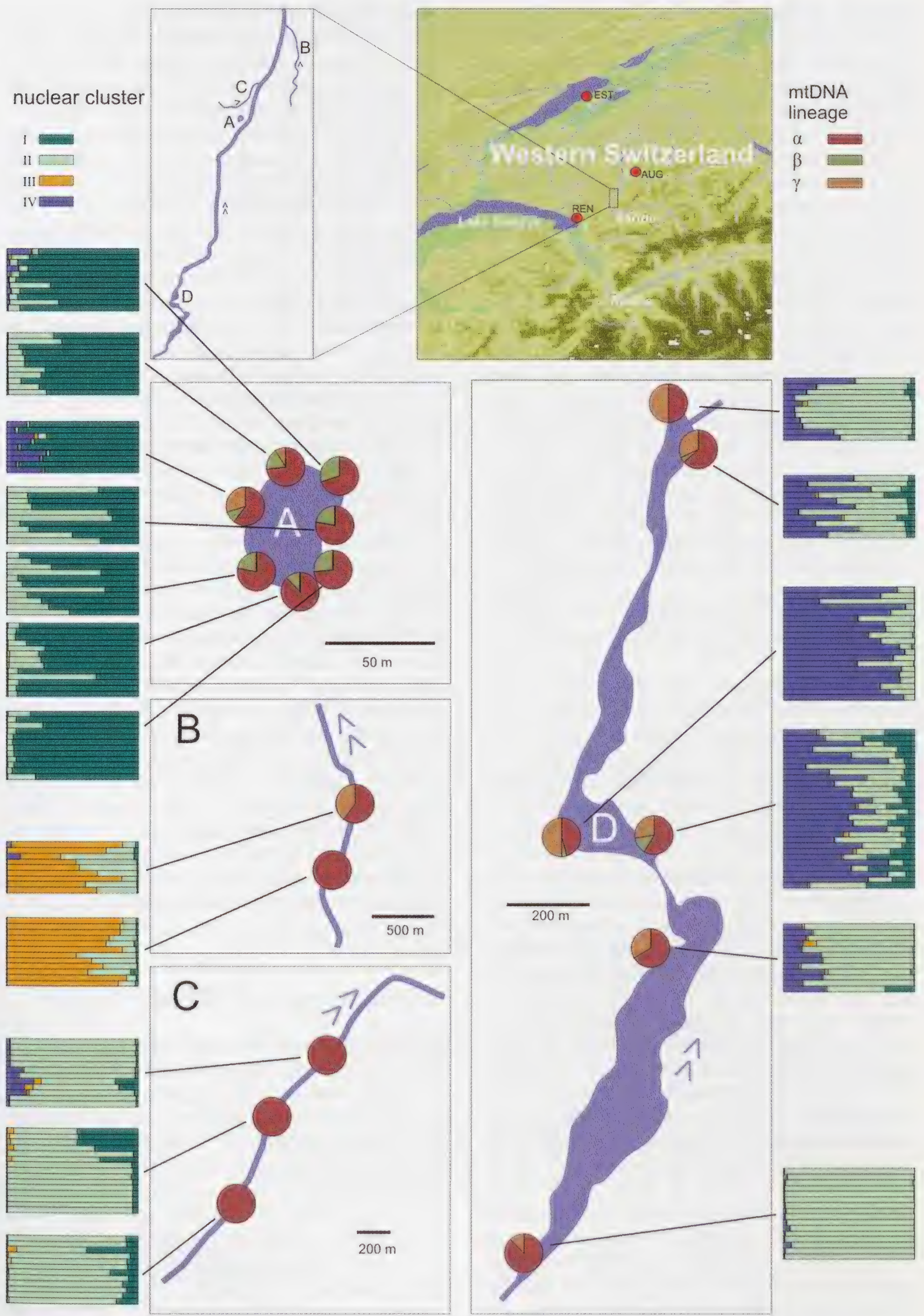
We conducted a series of studies that parameterised computer simulations to estimate the life time dispersal based on daily movements. This required knowledge of (i) the frequency distribution of daily movement distances, (ii) position preference in the habitat, (iii) dependence of movement directions on previous movement and (iv) dependence of positions between individuals (Pielou, 1969). Additionally, the seasonal water temperature is an important factor for the activity of freshwater snails and was taken into account.

Pond Set-up and Recording of Daily Net Movement Distances: To obtain a distribution of daily movement distances, we set up an artificial pond of 4 x 4 m in size. The study was conducted in June 2010, during which the mean daily maximum temperature was 18.8°C. We used tap water to fill the pond initially to 20 cm depth and inoculated it with 50 litres of natural pond water. As substratum, we inserted a 2 cm layer of sieved and dried local loam. We let the pond settle for two weeks, during which it was naturally filled up by rainwater to a depth of 30–35 cm and accumulated sufficient debris as a food source for the snails. Thirty individually marked adult snails > 10 mm from a laboratory stock population were released in the middle of the pond. After an acclimatisation period of one week, we recorded the position of living individuals every 24 hours relative to one corner of the pond for a period of 21 days. The daily net dispersal distances between two records were calculated as straight lines. We also recorded the dispersal directions and their changes between successive days. Individuals not surviving the measurement period were discarded from the analysis. The distribution of daily net movements over the study period was fitted to an exponential function.

Testing for a Preferred Position in the Habitat: Observations in the field and during the study suggested that the snails tended to stay close to the rim. We therefore measured the distance of each snail to the closest shoreline on the last day of the survey, to compare the observed distribution with that expected under a computer simulated movement model. For this simulation, a virtual snail was set at a random position within a virtual 4 x 4 m pond. A random variate was drawn from the observed daily net movement distribution and assigned to a random direction. If a snail would have crossed the rim of the pond with a movement in the simulation, it was reflected at the rim with the opposite direction and moved on the remaining distance. This was repeated 21 times for each snail and after the last move, the distance to the closest rim was recorded. The simulation was run 1,000 times for 29 snails.

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FIG. 1: Spatial distribution of the sampling sites along the Saane valley in Switzerland (square) and three known neighbouring populations (red dots). The position of the 18 sampling sites within the water bodies (labelled A–D) are indicated by coloured circles representing the fraction of the mtDNA lineages (α , β , γ), together with the cyto-nuclear composition of the individuals sampled. Vertical bar represents a single individual. Column shows the estimated nuclear composition of the multilocus microsatellite genotype (dark green = cluster I, light green = cluster II, yellow = cluster III, blue = cluster IV) as indicated by STRUCTURE.



Test for Independence of Previous Moves and of Individual Positions: Independence of movement directions from previous moves was tested by comparing the number of changes in consecutive movement directions to random expectations with a χ^2 test.

To test whether the snail's positions (and thus the movements) were independent from each other or whether they tended to aggregate or avoid each other, we recorded the average distance between the snails after 21 days and compared it to the expected distribution under a movement simulation model. We used basically the same simulation model as described above with two modifications. First, as previous results indicated that the snails moved almost exclusively along the rim of the pond, we considered only the distance vectors parallel to the rim from the observed daily net movement distribution. Second, because consecutive moves were not independent from previous moves, direction changes were randomly assigned according to the observed direction-change probability distribution. The average distance along the rim between all snails was recorded after 21 days. The simulation was run 10,000 times for 29 snails.

Temperature Dependence of Activity: To infer the temperature dependence of activity in *Radix*, individually marked snails ($n = 10$) of each of two laboratory stock populations were put in a 30 litre glass aquarium filled with aerated water in a climate chamber (one aquarium for each stock population). Food ad libitum (powdered TetraMin fish-food, Tetra) was provided. We increased the ambient temperature of the chamber from 2°C to 34°C in steps of 2°C/h. When the next temperature level was reached, which took about one hour, the temperature was held constant for the measurement of individual movements for one hour. Total distances covered in this hour by each individual were recorded. Movement per hour was averaged over both investigations. To establish the relationship between activity and water temperature, we calculated a linear regression between movement (dependent variable) and temperature (independent variable).

Modelling Life-Time Dispersal: The results of the simulations described above were used to design and parameterise a life-time dispersal model for *Radix*. It was assumed that the snails have a life span of approximately 260 days. This matches field observations of an

annual life cycle (Dillon, 2000) and equals the number of the days in the River Saanen Valley with a mean temperature above 5°C, excluding the time for hibernation. To simulate the life time dispersal distance of a snail in a two dimensional habitat of unrestricted size, it was initially placed at position zero. A random variate from the exponential function of the observed daily movement distance distribution along the shore line was drawn. To take the temperature dependence of movement activity in *Radix* into account, the assumed life time was divided into eight periods of 30 days (corresponding to the months March to November) and two shorter periods of ten days at the beginning and the end (each during February and December). The determined temperature-dependent activity function was then used to determine the correct drawn movement distance according to the average monthly mean temperature in the Saanen Valley. Climate data for the Saanen Valley were taken from <http://www.meteoschweiz.admin.ch>. Whether the direction of movement for the subsequent day changed or not was randomly determined according to the observed direction change probability. This procedure was repeated 260 times and the distance of the final position to the starting point of the snail recorded. We simulated 100,000 snails in total.

Wright (1946) calculated the neighbourhood size for a linear-distributed population in a two-dimensional habitat as $N = \sqrt{2} p \cdot \sigma \cdot d$, with σ being the variance of dispersal along the habitat from parents relative to their offspring at the same developmental stage and d as the observed density of breeding individuals. The strip length where parents potentially can originate from and no population differentiation can be expected thus equals about 3.55σ (Wright, 1946).

Indirect Estimation of Population Structure

Sampling Design: Sampling was conducted in a part of the river Saanen Valley, Switzerland. We searched for *Radix* populations in all natural and artificial water bodies in the valley between Montbovon (46.628°N, 7.044°E) and the Lac de la Gruyère (46.623°N, 7.101°E) and below 1,000 m altitude. Two hundred and seventy individuals from 18 sites were sampled. *Radix* were found in only four out of 35 water bodies: two small creeks, a gravel pit and a reservoir of the Saanen river (Table 1, Fig. 1). Along the creeks where *Radix* occurred, all adult snails (> 8 mm) that could be found were sampled

TABLE 1. Overview of the 18 sampled localities, the *STRUCTURE* clustering (A, B, C and D) with the exact coordinates (°N and °E), the age of the waterbody and the number of individuals sampled (N_t). Mitochondrial lineage present at each locality (α , β , γ). Genetic diversity was estimated by mitochondrial DNA marker COI for n individuals (N_{indmt}) with haplotype diversity (D_{hap}), mean Nei's gene diversity (D_{mt}) with standard deviation ($s.d.$) and by nuclear DNA markers (Salinger & Pfenninger, 2009) for n individuals (N_{indnc}). (N_a = number of alleles; D_g = mean Nei's gene diversity; $s.d.$ = standard deviation; H_o = heterozygosity observed; H_e = heterozygosity expected. The inbreeding coefficient (F_{IS}) was calculated for each sampling site and the selfing rate s were estimated by three methods: F_{IS} , g_2 and ML .

Locality	Waterbo- dy type	N_o	π°	age (years)	lineage	D_{hap}	D_{mt}	$s.d.$	N_{indmc}	N_a	$s.d.$	D_g	$s.d.$	$p.s.d$	H_o	H_e	F_{IS}	$s(F_{IS})$	$s(g_2)$	$s(ML)$
A	gravel-pit	46.54937	7.07865	~ 10					77											
A1		46.54943	7.07869	~ 10	α , β	0.200	0.004	0.002	11	2.25	1.21	0.348	0.258	0.159	0.332	0.521	0.362	0.000	0.018	
A2		46.54939	7.07872	~ 10	α , β	0.222	0.003	0.002	10	2.00	0.82	0.300	0.230	0.106	0.284	0.627	0.521	0.901	0.837	
A3		46.54933	7.07872	~ 10	α , β	0.200	0.004	0.002	15	2.50	1.50	0.341	0.251	0.114	0.328	0.652	0.538	0.000	0.051	
A4		46.54939	7.07872	~ 10	α , β	0.375	0.003	0.001	10	2.25	1.21	0.294	0.243	0.150	0.279	0.462	0.312	0.231	0.226	
A5		46.54931	7.07860	~ 10	α , β	0.200	0.003	0.001	11	2.38	1.13	0.379	0.209	0.155	0.361	0.571	0.416	0.535	0.506	
A6		46.54938	7.07852	~ 10	α , β , γ	0.250	0.004	0.002	9	1.88	1.17	0.381	0.272	0.215	0.360	0.403	0.188	0.320	0.416	
A7		46.54943	7.07857	~ 10	α , β , γ	0.182	0.003	0.001	11	2.13	0.41	0.314	0.250	0.046	0.300	0.847	0.801	0.764	0.756	
B	creek	46.55088	7.09522	> 10,000					21											
B1		46.55963	7.09479	> 10,000	α	0.154	0.000	0.000	14	2.00	0.49	0.363	0.181	0.205	0.350	0.414	0.209	0.359	0.140	
B2		46.56065	7.09578	> 10,000	α , γ	0.600	0.005	0.002	7	1.63	0.45	0.239	0.210	0.110	0.221	0.502	0.392	0.764	0.576	
C	creek	46.55066	7.07707	> 10,000					39											
C1		46.55093	7.07726	> 10,000	α	0.125	0.001	0.001	13	2.13	1.50	0.251	0.304	0.087	0.241	0.639	0.552	0.764	0.633	
C3		46.55196	7.07832	> 10,000	α	0.200	0.000	0.000	14	1.85	0.50	0.205	0.200	0.135	0.197	0.315	0.180	0.645	0.545	
C5		46.55326	7.07938	> 10,000	α	0.083	0.000	0.000	12	3.38	0.50	0.235	0.275	0.099	0.225	0.560	0.461	0.442	0.568	
D	reservoir	46.49670	7.05196	~ 100					98											
D1		46.49459	7.05167	~ 100	α , γ	0.500	0.006	0.002	11	4.38	2.00	0.443	0.200	0.288	0.420	0.314	0.026	0.000	0.019	
D2		46.50536	7.05272	~ 100	α , β , γ	0.304	0.008	0.002	13	4.38	2.60	0.560	0.165	0.317	0.539	0.412	0.095	0.071	0.071	
D3		46.50675	7.05248	~ 100	α , γ	0.800	0.014	0.004	10	3.50	1.92	0.500	0.124	0.306	0.474	0.354	0.048	0.419	0.379	
D4		46.48808	7.04774	~ 100	α , γ	0.375	0.002	0.001	16	2.88	1.13	0.445	0.223	0.263	0.430	0.388	0.125	0.209	0.319	
D5		46.49727	7.04901	~ 100	α , β , γ	0.363	0.007	0.002	20	4.50	2.00	0.541	0.179	0.313	0.527	0.406	0.093	0.291	0.199	
D6		46.49707	7.05188	~ 100	α , β , γ	0.270	0.007	0.002	28	5.13	2.85	0.586	0.187	0.359	0.576	0.377	0.177	0.143	0.052	
all							0.005	0.001	235	4.39	2.36	0.389	0.120	0.214	0.486	0.510	0.346	0.332	0.153	

along a stretch of 5 m, every 50 m. In the gravel pit, all adult snails were sampled and pooled per 10 m shoreline. If the density was high, a maximum of 22 adult snails were taken per section. At the reservoir, all accessible parts of the shoreline were searched and 11–30 snails were sampled at places where *Radix* were present. Individuals were immediately preserved in 100% ethanol. The distances between sampling sites were measured along the shoreline (Table 2) for comparison to the neighbourhood size calculated from the simulation of lifetime dispersal.

Microsatellite and Mitochondrial Haplotype Analysis: DNA was extracted using a glass fibre DNA extraction method following the protocol of Ivanova et al. (2006). About 1 mm³ of alcohol-preserved snail tissue was mixed with vertebrate lysis buffer containing 100 mM NaCl, 50 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 0.5% SDS and 20 mg per ml proteinase K. Samples were incubated over night at 56°C. After digestion, samples were mixed with binding buffer (BB), composed of 6 M guanidinium thiocyanate, 20 mM EDTA, 10 mM Tris-HCl (pH 6.4), 4% Triton X-100, transferred into a Pall1 glass fibre plate (GF plate, AcroPrep™), placed on top of a 2 ml square well block and centrifuged to bind DNA to the GF membrane. DNA washing was carried out in two steps: (1) Protein wash buffer: BB and EtOH 96%, (2) Wash buffer: 60% EtOH, 50 mM NaCl, 10 mM Tris-HCl (pH 7.4), 0.5 mM EDTA (pH 8.0). After washing, residual ethanol was evaporated by incubating the GF plate at 56°C for about 30 minutes. DNA was eluted by incubating each GF membrane with 50 µl of prewarmed (56°C) ddH₂O followed by centrifugation using a 96-well collection plate.

Cytochrome oxidase subunit I (COI) fragments were amplified using polymerase chain reaction (PCR) performed with Invitrogen Taq DNA polymerase and universal primers (Folmer et al., 1994). Sequencing reactions were performed using the ABI Prism Big Dye terminator kit (Perkin-Elmer, Norwalk, CA, USA). Sequenced fragments were read on an ABI Prism 3730 automated capillary sequencer (Applied Biosystems).

All snails were genotyped for eight highly polymorphic microsatellite loci (Salinger & Pfenninger, 2009). Multiplex Microsatellite amplification was carried out using the Quiagen Type-it™ microsatellite PCR Kit with fluorescent dye labeled forward primers (Salinger & Pfenninger, 2009). PCR products were

separated and visualized on an ABI Prism 3730 sequencer (Applied Biosystems) with GeneScan™ 500-LIZ™ size standard (Applied Biosystems). Microsatellite allele lengths were recorded using GENEMAPPER 4.0 software (Applied Biosystems).

Genetic Variability: Mitochondrial sequences of the COI-fragment of 482 bp length were aligned using ClustalW (Thompson et al., 1994) as implemented in MEGA 4.0 (Tamura et al., 2007) and corrected by eye. We used this alignment to calculate a parsimony haplotype network in TCS 1.21 (Clement et al., 2000). We nested haplotypes in a hierarchical fashion as described by Pfenninger & Posada (2002). Identification of the sampled snails as *R. balthica* was done by comparing the respective barcodes to the reference sequences of Pfenninger et al. (2006; GenBank Accession no. DQ980030–DQ980193) using a neighbour-joining tree and monophyly with the previously identified haplotypes. This was necessary, because other *Radix* species are present in the region and specific identification is only possible with molecular markers (Pfenninger et al., 2006). Mitochondrial genetic distances among the water bodies were calculated as Nei's standard genetic distances (D_{mt} ; Nei, 1972) with MEGA 4.0 (Tamura et al., 2007).

We used GenePop (Raymond & Rousset, 1995a) to determine allele and genotype frequencies of the microsatellite markers, for the different sampling sites, the four water bodies and the entire sample. To investigate population differentiation, F_{ST} was calculated using *FSTAT* 2.9.3.2 (Goudet, 1995). *FSTAT* was also used to estimate the expected (H_E) and observed heterozygosities (H_O) for each sampling site and to test for deviation from Hardy-Weinberg Equilibrium among sampling sites within water bodies (1,000 permutations, two-sided test). Bonferroni correction for multiple tests was applied to each of these tests. Nuclear genetic distances among the water bodies (D_G ; Nei, 1972) were calculated using Genetix 4.04 (Belkhir et al., 1996–2004). The microsatellite data were also subjected to a factorial correspondence analysis with Genetix 4.04 (Belkhir et al., 1996–2004) to visually explore the distribution of genetic variation.

Sensitivity Test: Because our data set contained sampling sites with relatively few individuals, we conducted two initial simulation studies to assure that observed deviations from the null hypothesis of no population structure are

not due to sampling artefacts. First, we simulated 300 times a truly panmictic population of 200,000 individuals with a diploid genotype of eight independent loci with the same possible number of allelic states as observed in our sample of natural populations, respectively, using EASYPOP (Balloux, 2001). The mutation rate was set to 0.05 and a model with mutations equally likely to occur at any of K possible sites (KAM) was chosen. After 1,000 generations, a dataset from the final generation of 235 individuals was generated, divided into 18 groups of the same size as our sample of natural populations. A second dataset was generated almost identically according to the sampling parameters at sampling site A. The number of individuals was set to 77 and the number of groups was set to 7.

The resulting datasets were tested for deviation from the null hypothesis of no population structure with an AMOVA (Analysis of Molecular Variance, 10,000 replicates) in Arlequin 3.11 (Excoffier et al., 2005).

Inference of Population Structure: Assignment of individuals to genotype clusters without a priori classification was performed with STRUCTURE (Pritchard et al., 2000). All MCMC runs were repeated five times for each value of K ($K = 1$ to $K = 20$) for 1,000,000 generations with 50,000 burn-in steps, applying the admixture model. We also used a new model developed for STRUCTURE that integrates information about sampling locations. This can be useful if the cluster identification without a priori information would not suffice to detect clusters in a dataset (Hubisz et al., 2009). We used the same settings as in the model without the location-prior. Both models were analysed using the maximum $\text{LnP}(D)$ value. $\text{LnP}(D)$ is the log likelihood of the observed genotype distribution in K clusters.

F_{ST} among water bodies was calculated with Genetix 4.04 (Belkhir et al., 1996–2004). An exact test of population differentiation (Raymond & Rousset, 1995b) was performed using Arlequin 3.11 between all pairs of sampling sites within the respective water bodies, with 1,000,000 steps of Markov Chain length and 1,000,000, dememorization steps.

Isolation by Distance: We tested for Isolation-by-Distance among sampling sites using a Mantel's test (Mantel, 1967). A correlation between $F_{ST} / (1 - F_{ST})$ and the natural logarithm of pair-wise geographical distances was obtained using the ISOLDE

program of the GENEPOP package (Raymond & Rousset, 1995a; Rousset, 2008).

Estimation of Selfing Rates: Genotype frequencies at codominant marker loci may convey information on mating systems (David et al., 2007). The classical approach is to measure heterozygote deficiencies and obtain the selfing rate s from $F_{IS} = s / (2-s)$, assuming inbreeding equilibrium. A drawback of this estimate is that heterozygote deficiency may not only be due to selfing. Biparental inbreeding and partial dominance as well as such technical artefacts as null alleles also increase the proportion of observed homozygotes. Therefore, in addition to this classical approach, we used a method implemented in RMES (David et al., 2007) to derive estimates of $s(g_2)$, based on a point estimate of the second-order heterozygosity disequilibrium and a maximum likelihood of the whole distribution based approach of $s_{(ML)}$. These estimates are independent of F_{IS} and less influenced by technical biases.

Assignment Tests: To compute the probability of each individual being derived from external populations or being a resident (i.e., not a first-generation migrant) in the Saanen Valley, we used GENECLASS2 (Piry et al., 2004). As a reference dataset we used 80 populations, covering the approximate species range, from a previous study on *R. balthica* (Pfenninger et al., 2011). This software computes genetic assignment statistics to assign or exclude reference populations as the origin of individuals on the basis of multilocus genotype data. It is thus also possible to obtain direct estimates of dispersal through the detection of immigrant individuals (Rannala & Mountain, 1997; Paetkau et al., 2004). For the assignment test and the test for first generation migrants we selected the likelihood ratio $L_{\text{home}} / L_{\text{max}}$ and a Bayesian approach by Baudouin & Lebrun (2001) with an assignment threshold of scores of 0.01 and tested for probabilities using a simulation algorithm by Paetkau et al. (2004) with 100,000 simulated individuals and an alpha error set to 0.05. This approach provides a probability estimate of exclusion from or inclusion in a population, given only the genotype of the animal in question, and estimates of the allele frequency of the population in question. This simulation approach is sensitive to sample size and the degree of genetic differentiation among the populations. The significance of results was estimated with Monte Carlo re-sampling techniques as implemented in GENECLASS2 (Paetkau et al., 2004).

RESULTS

Movement Dispersal Patterns

During the 21 days of the study, 402 moves were recorded from 27 snails. Daily distances ranged from 0.01 to 3.86 m, with a median of 0.43 m. Distances covered in 21 d ranged from 3.22 m to 23.02 m, with a mean of 13.60 ± 4.96 m (mean $\pm$ s.d.). The frequency distribution of daily dispersal distances fitted a function with exponential decay ($y = 85.7 e^{-0.23x}$; $r^2 = 0.88$, $p < 0.001$, $n = 1,000$).

The observed average distance between individuals after 21 days was 5.25 ± 3.88 m (mean $\pm$ s.d.). Average inter-individual distance from a random movement simulation was 5.53 ± 3.95 m (mean $\pm$ s.d.). The observed value falls well within the 95% confidence interval. The null hypothesis of random distribution could thus not be rejected.

Movement along the rim was preferred over movement away from the rim ($\chi^2 = 47.12$; $n = 395$; $d.f. = 3$; $p < 0.001$; comparison with a uniform distribution). The snails lived and moved almost exclusively close to the rim of the artificial pond. Their observed distribution relative to the rim was significantly different from the expected distribution obtained from

a random movement simulation. Directions of second moves were dependent on direction of the first moves ($\chi^2 = 22.69$; $n = 365$; $d.f. = 2$; $p < 0.001$; comparison of deviation from first move with a uniform distribution). Snails maintained directions on consecutive days in 62% of cases. Random movement could thus with respect to direction not be assumed.

Movement of *R. balthica* was strongly temperature dependent. A linear regression for the distance moved per hour against water temperatures was highly significant (Fig. 2).

Computer simulated mean effective lifetime dispersal in a two-dimensional habitat was 48.25 m (s.d. = 35.75 m) in 260 d. Minimum simulated effective lifetime dispersal distance was 0.11 m and maximum distance was 229.62 m.

Direct Estimation of Population Structure

The standard deviation of life time dispersal was 35.75 m. The spatial extent of a neighbourhood was thus 126.9 m of shore line. If we compare this to the distances between the sampling locations (Table 2), we do not expect population differentiation at least among sampling points in water body A and between sampling points D2 and D3.

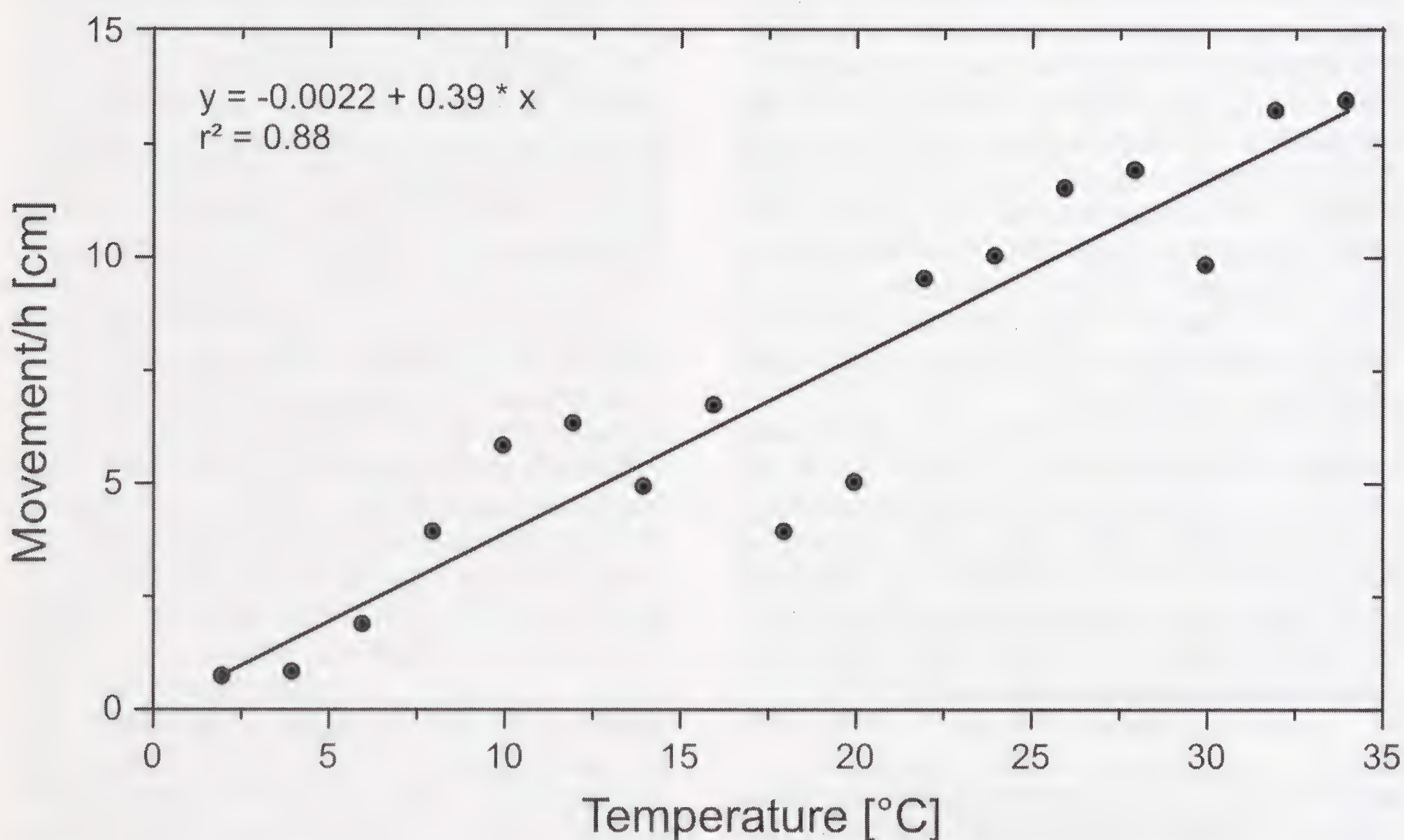


FIG. 2. Linear regression of *R. balthica* movement per hour against water temperature. Movement per hour are averages measured from 10 snails. The regression was highly significant ($p < 0.0001$).

TABLE 2. Distances in meters between the sampling points along the shoreline within the water bodies. Asterisks show significance level of an exact test of population differentiation (*p < 0.05; **p < 0.01; ***p < 0.001) between all pairs of samples within the water bodies.

	A1	A2	A3	A4	A5	A6	A7	B1	B2	C1	C2	C3	D1	D2	D3	D4	D5	D6
A1																		
A2	25																	
A3	39*	14																
A4	53	28	14															
A5	75	50	36	22														
A6	48	76	67	53	31													
A7	24*	48**	63**	76*	56*	25*												
B1								418										
B2								418										
C1										342	587							
C2										342	248							
C3										587	248							
D1													1748	1621	939*	482**	2256**	
D2													1748	125	2607*	1286***	1250**	
D3													1621	125	2567*	1161*	1364*	
D4													939*	2607*	2567*	1390*	1404*	
D5													482**	1286***	1161*	1390*	2467	
D6													2256**	1250**	1364*	1404*	2467	

Incidence of *Radix balthica* in the Sampling Region

Despite the presence of many seemingly suitable water bodies in the Saanen Valley, *Radix* snails were found only in a small fraction of them (4 out of 35). These tended to be the larger ones, although the species was absent from one of the larger gravel pits in the immediate vicinity of water body A.

Mitochondrial Haplotype Variability

At least 482 bp of the COI-gene fragment were obtained and manually aligned for 236 individuals from the Saanen Valley. All were identified as *R. balthica* by comparing the sequences to the reference sequences (data not shown). There were 28 polymorphic sites in our sample and 19 haplotypes (GenBank, accession no. GU735965-GU736200). Haplotypes were designated according to the nomenclature of Cordellier & Pfenninger (2009). Statistical parsimony analyses clustered these haplotypes into three lineages (α , β , γ , Fig. 3). Lineage α included 9 haplotypes from more than half the individuals analyzed ($n = 169$), β comprised 4 haplotypes from 23 individuals and γ included 6 haplotypes from 34 individuals. The distribution

of haplotype lineages among sampling sites is shown in Figure 1. Genetic diversity within lineages was between 0.4 and 5.9% and genetic divergence among lineages ranged from 0.1 to 7.1%. The genetic diversity at each sampling site and within each water body was measured by two estimators, haplotype diversity (D_{hap}), which ranged from 0.08 to 0.8, and genetic diversity (D_{mt}), which ranged from 0 to 14% between sampling sites with a mean of 5% differentiation (Table 1).

Nuclear Microsatellite Variability

Two-hundred-and-thirty-five individuals were successfully genotyped at 8 microsatellite loci. The number of alleles per locus ranged from 4 to 16, with an average of 4.4 (Table 1). The mean number of alleles (N_a) ranged from 1.85–5.13; Nei's mean genetic diversity had values from 0.2 to 0.59 with an overall mean of 0.39. All microsatellite loci showed consistent heterozygote deficiency with an overall observed heterozygosity of 21.4% and an expected heterozygosity of 48.6% for the sampling sites. Values of expected heterozygosity (H_e) ranged from 0.19–0.58 and observed heterozygosity (H_o) from 0.1–0.36. Detailed values are shown in Table 1. Significant devia-

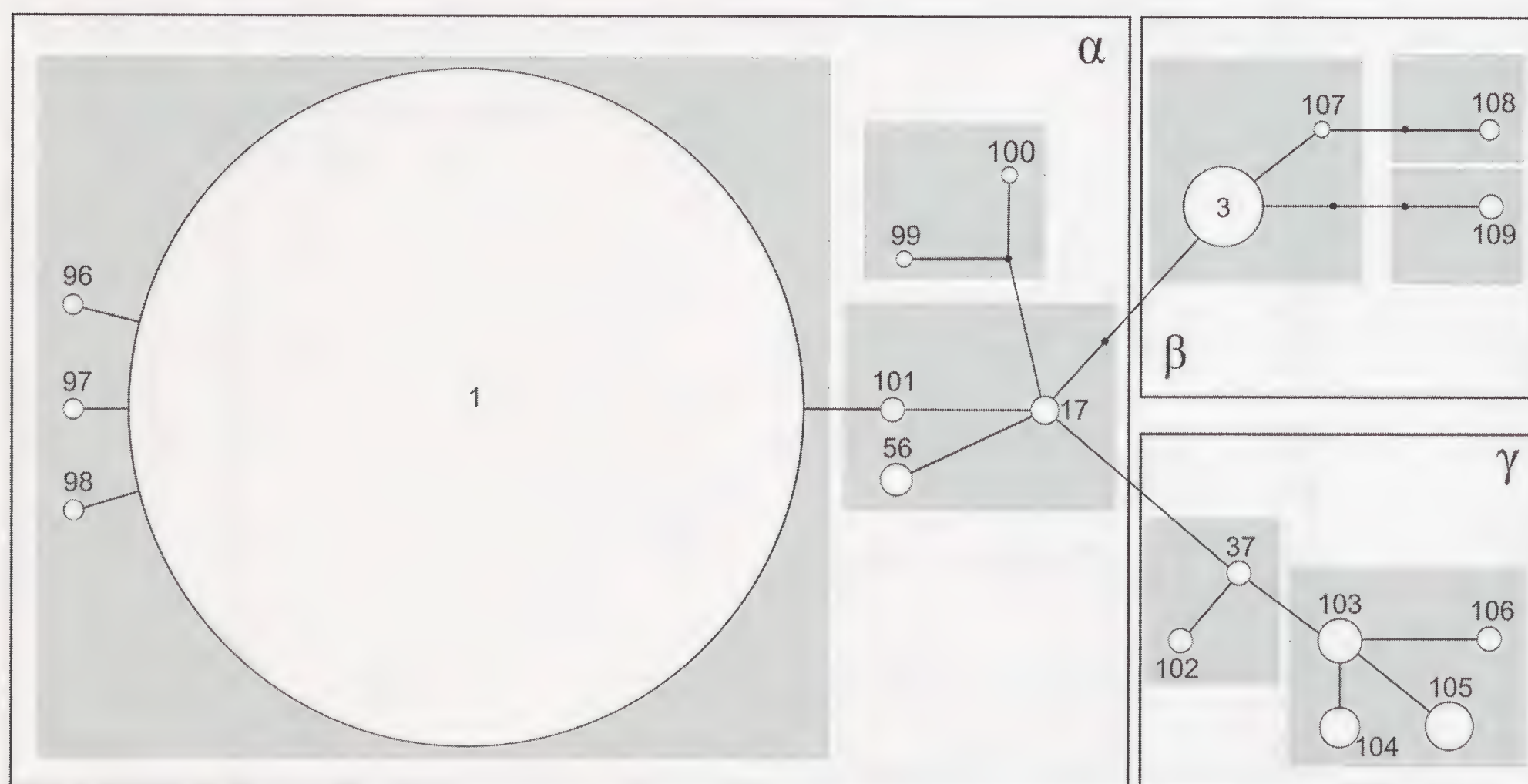


FIG. 3. Minimum spanning network of *R. balthica*. Each circle represents a haplotype, its size being proportional to the frequency of a certain haplotype. Connecting lines represent one mutational step. Small black circles depict haplotypes that were not sampled. The lineages are named α , β , γ .

TABLE 3. The grouping of the nuclear genotypes into four clusters (I, II, III and IV) is based on the *K* from the *STRUCTURE* analysis. Lineage α , β and γ refer to the inferred mitochondrial lineage. The *RXC* exact contingency table test on independence was significant (*d.f.* = 6, χ^2 = 16.6, *p* < 0.001).

	α	β	γ
I	57	8	5
II	41	6	4
III	8	6	8
IV	39	1	13

tions from Hardy-Weinberg equilibrium were observed in nearly half the loci in the sampling points, even after Bonferroni’s correction. We attribute this systematic deviation to a mixed mating system common in pulmonates (Jarne & Stadler, 1995) and described for *R. balthica* (Salinger & Pfenninger, 2009).

Cyto-Nuclear Association Test

There was a weak, albeit significant association of the two nuclear clusters (inferred from the ΔK) with mitochondrial haplotype lineages (*N* = 196, *p* < 0.001, χ^2 = 16.6, *d.f.* = 6, exact 4x3 *RXC* contingency test). Slightly less individuals of nuclear cluster I contained lineage γ haplotypes than expected by chance. The same was true for nuclear cluster III individuals with lineage α haplotypes (Table 3).

Sensitivity Test

The simulated datasets indicated that the actual number of loci used, their variability and the applied sampling design were capable of correctly keeping the null hypothesis of no

population differentiation if it is true. This was true in 291 out of 300 tested cases, for the full dataset. For the reduced dataset, equivalent to water body A, this was true in 287 out of 300 tested cases, corresponding in both cases to a type I error of less than 0.05.

Inference of Population Structure

Applying a location prior to the *STRUCTURE* analysis found the highest mean likelihood value for *K* = 4. Seventy individuals were attributed to the first cluster (I), 51 to the second (II), 22 to the third (III), 53 to the fourth (IV). However, even though the number of clusters found corresponded to the number of water bodies examined, there was no clear association between them (Fig. 1).

The overall *F*_{ST} among all sampling sites was 0.15, ranging from 0.08 to 0.25. Results of the test for exact population differentiation within the water bodies showed highly significant differentiation between several sampling points (Table 2).

We found no pattern of overall isolation-by-distance (*r*² = 0.0005, Mantel test *p* = 0.491).

Population Assignment Test

Applying the simulation approach, 213 out of 235 animals (91%) were genetically assigned as residents rather than immigrants. Assignment was probable to three outside populations for 13 individuals to a neighbouring population in northern Switzerland (AUG – 46.615°N, 7.181°E) and seven and two individuals to either of two populations in southern Sweden (SEM – 57.155°N, 16.419°E; EDA – 59.834°N, 17.878°E).

Testing for first generation migrants highlighted a single individual with most likely external origin in AUG. Eight individuals identified as first

TABLE 4. An analysis of molecular variance (AMOVA) for microsatellite data for the 4 clusters estimated with *STRUCTURE* (*K* = 4).

	<i>d.f.</i>	Sums of squares	Variance components	% of variation	<i>p</i> -value
<i>STRUCTURE</i> clustering = 4					
Among water bodies	3	79.1	0.23	13.56	0.001
Within water bodies	466	690.5	1.48	86.44	0.001
Total	469	769.6	1.71		

generation migrants, however, were assigned to other sampling sites within the study. Four individuals from A were assigned to C, both one individual from B was assigned to D and C respectively and three individuals from D were assigned to C.

Estimation of Selfing Rates

The inbreeding coefficient F_{IS} of the sampling sites ranged from 0.31 to 0.57 with an overall mean of 0.51. The selfing rate s estimated from F_{IS} ranged from 0.02 to 0.8, the overall mean was 0.34. The selfing rate derived with *RMES* (David et al., 2007) differed from the former estimate not so much in the mean, rather in the variance, as $s(g_2)$ ranged from 0 to 0.9 with an overall mean of 0.33. The Maximum Likelihood estimate of $s_{(ML)}$ ranged from 0.01 to 0.84 with an overall mean of 0.15.

DISCUSSION

Observed Local Population Structure Does Not Match Expectations from Active Dispersal

Estimating dispersal from observational data (Cameron & Williamson, 1977) or in combination with computer simulation has been successfully applied before in several studies on land snails (Baur & Baur, 1993; Pfenninger et al., 1996). These studies showed that simulated dispersal was similar to dispersal actually measured in natural habitats which justified its application in our study.

From active dispersal alone, we would have expected a homogeneous population within water body A and isolation by distance in water body C because: (i) the estimated neighbourhood where no differentiation may be expected comprises ~130 m of shoreline, which approximately corresponds to the shoreline of water body A, and (ii) our marker system would probably not be able to distinguish even a multiple of this measure. Nevertheless, sensitivity analysis of our data proved their suitability for correctly supporting the null hypothesis of no differentiation. However, the population differentiation inferred by nuclear and mitochondrial markers was much larger than expected (Table 4). There are several possible explanations for these discrepancies. First, the direct estimate might be exaggerated due to our study setup because (i) natural water bodies with diverse habitat structure might reduce the actual

neighbourhood size range due to preference of certain spots (Bovbjerg, 1968), and (ii) the density in the artificial pond might have been too high, which may have lead to crowding effects. However, since snails in most water bodies occurred in much higher abundances than in the study and were uniformly distributed along the shoreline (personal observation MP), these two issues should be of minimal importance. Second, the parameters from which neighbourhood size was calculated could have been derived from incorrect assumptions. For example, measurement of the temperature dependence of activity might not translate in a linear fashion to the field. Snails were kept in different temperature regimes for only one hour, so the metabolism might have lagged behind the actual temperature or the recorded distances might just show acclimatisation activity (Bailey & Johnston, 2005); for example, the attempt to escape high water temperatures that would have seized after a while. However, the linear response to temperature shifts argues against this latter possibility. Rather the relative correction of the dispersal distances measured in the field avoided the introduction of a systematic lag bias. Also a non-linear dependence of air temperatures (as reflected in the climate data) to water temperature would have biased the simulation results. However, except for water bodies influenced by geothermic or anthropologic activity, temperatures of surface water bodies, such as those observed here usually correspond in a linear fashion to air temperature.

The use of a *Radix balthica* population from outside the valley for the dispersal study might have biased the estimate if dispersal activity is population specific (Terblanche et al., 2009). Heritable variation in dispersal activity cannot be excluded; however, it is highly unlikely that differences on the order of magnitudes necessary to explain the discrepancy exist among the populations used for the direct approach and those observed in the field.

Third the mixed mating system could influence the population structure. High selfing rates, which ranged from 0 to 0.9 (mean 0.33; Table 1) in our study, should increase local inbreeding, thereby strongly decreasing the actual neighbourhood range (especially in the case of complete selfing) and thus lead to stronger spatial differentiation. However, in this case we would expect a pronounced isolation-by-distance pattern within water bodies at dispersal-drift equilibrium, which is not the case.

To explain the observed population structure based on neighbourhood range alone, the neighbourhood must not exceed a few square meters, which seems very unlikely, given the observational data. Even though we cannot be sure that we did not overestimate the neighbourhood range, lack of active dispersal is probably not sufficient to explain the structure observed. A non-equilibrium situation therefore remains as the most probable explanation.

Independent colonisation of the valley from several sources in combination with metapopulation dynamics and the mixed mating system may provide the processes causing the observed pattern. The haplotype and nuclear diversity found in the valley encompassed a substantial fraction of the diversity in the entire species range (Cordellier & Pfenninger, 2009), indicating colonisation of the valley from different sources. Indeed, the deviation of observed data from expectations results mainly from the presence of different nuclear clusters/mitochondrial lineages at certain sampling points within water bodies (Fig. 1). For example, sampling point A7 is differentiated from the other sampling points in water body A. This part is inhabited by a different lineage, indicating two independent colonisation events.

The weak albeit significant association of mitochondrial lineages with nuclear clusters indicated that there is either high ongoing gene-flow, constantly supplying individuals from the same populations or that the mixed mating system retards complete mixing of individuals from several immigration events in the past. The low estimate of first generation migrants both from within the valley and from the entire species range argues for the latter scenario.

Absence of the species from many apparently suitable water bodies during the sampling program, but subsequent colonisation of some (personal observation MP and TH) indicates a substantial metapopulation dynamic, which may influence population structure strongly. Metapopulation dynamics with frequent extinction and recolonisation events are common in other freshwater snails (Viard et al., 1997; Charbonnel et al., 2002; Facon & David, 2006). Since *Radix* shells are very thin-gauge, it is not possible to find empty shells which could give a hint at past occupations from other water bodies in the valley.

While the snails are not able to cross land barriers, passive dispersal must be assumed. For freshwater snails in particular, waterfowl have been suggested as dispersal vectors (Malone,

1965; Boag, 1986; Bilton et al., 2001). This is even more likely since land snails can survive passage through a bird's gut (Wada et al., 2011) and freshwater snails passage through a fish's gut (Brown, 2007). However, direct data on the qualitative and quantitative impact of bird-mediated dispersal is lacking and thus urgently needed to fully understand the population dynamics of freshwater invertebrates.

The fine-scale population structure here inferred by nuclear and mitochondrial markers thus reflects the species range-wide structure (Pfenninger et al., 2011), shaped by processes such as long-range dispersal, large-scale metapopulation dynamics and low levels of local passive dispersal. Active dispersal had, even in smallest water bodies, a nominal impact on local population structure. Furthermore, the hereby formed structure is preserved by the mixed mating system. We found no evidence that local processes to influence the observed structure.

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TH and MS performed the molecular work, analysed the data and drafted the manuscript, AP helped with the simulation models, MP designed the study, did the fieldwork, programmed the simulation models, analysed the data and contributed to writing the manuscript. All authors have approved the final version of the manuscript.

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A NEW CRYPTIC SPECIES OF CARDITID BIVALVE FROM THE GULF OF CALIFORNIA (MOLLUSCA, BIVALVIA, ARCHIHETERODONTA, CARDITIDAE)

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ABSTRACT

Carditamera bajaensis, new species, is described from semi-infaunal specimens collected in the intertidal zone in the Golfo de California, Baja California Sur, Mexico. The new species resembles *Carditamera affinis* (G. B. Sowerby I, 1833), the only valid *Carditamera* species known from within the Golfo de California, with which it has been mistaken, but it differs in shell structure and most conspicuously in life mode – semi-infaunal for ***C. bajaensis*** versus byssally attached to hard substrata for *C. affinis*. Haplotype networks constructed from two mitochondrial genes (16S rRNA and cytochrome *b*) and one nuclear gene (internal transcribed spacer 2) indicate a clear genetic break between *C. affinis* and ***C. bajaensis***, as suspected initially due to their different modes of life and shell morphology. This pair of species, *C. affinis* and ***C. bajaensis***, overlapping in distribution yet genetically distinct, possibly indicate ecological speciation.

Key words: Cryptic species, Carditidae, *Carditamera*, Baja California, Archiheterodonta, haplotype networks.

INTRODUCTION

Western North America has a particularly species-rich marine bivalve fauna (Coan et al., 2000; Coan & Valentich-Scott, 2012), especially with regard to members of Carditidae. Carditid bivalves are exclusively marine, predominantly infaunal (though some byssate forms occur), suspension-feeders that lack siphons. Carditids are one of the neglected larger families of bivalves; the last genus-level systematic treatment of the Carditidae was given by Chavan (1969), and only 50 species were ascribed to this group by Boss (1982). Recently, 92 species were illustrated by Huber (2010), who recognized approximately 140 species in the family.

Carditidae are postulated to have originated in the Ordovician and have been recorded as early as the Devonian (Chavan, 1969), though the lineage diversified much later in the Cretaceous (Slack-Smith, 1998). The development and reproductive biology of Carditidae was examined by Dall (1902). Most species are brooders, in which juveniles are retained within the body cavity of the female until the process of secretion of the calcified shell has commenced,

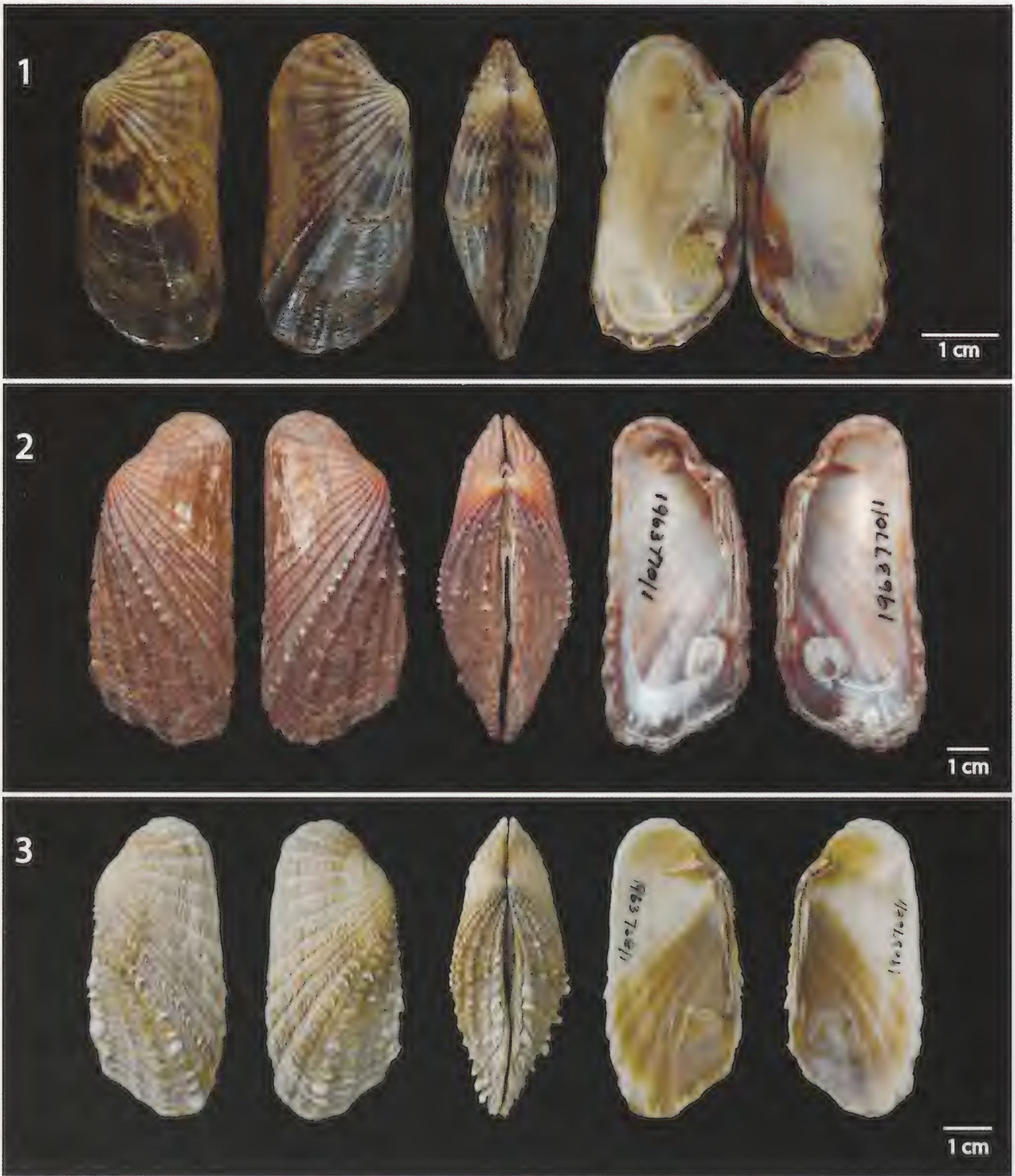
along with the complete formation of the prodissoconch (Mikkelsen & Bieler, 2008). Though carditids predominantly inhabit shallow water, several members, such as the mainly Arctic and boreal *Cyclocardia*, have been collected at depths of up to 1,707 m (Dall, 1902).

Phylogenetically, Carditidae clusters with three other basal heterodont bivalve families – Astartidae, Crassatellidae, and Condyllocardiidae – constituting the well-supported clade Archiheterodonta (Taylor et al., 2007; Giribet, 2008). Though no condyllocardiid species has been yet included in a molecular phylogenetic analysis, morphologically Condyllocardiidae has been placed within this clade (Middlefart, 2002). Astartidae, a smaller family, includes four living genera with approximately 40 species that inhabit Arctic to temperate waters (Huber, 2010). Crassatellidae, a moderately sized family, includes 13 living genera containing approximately 85 species and has a global distribution, though most crassatellids are found in tropical and subtropical regions (Huber, 2010). Condyllocardiidae, a larger family, includes 21 living genera and about 150 species distributed globally, though little is known about the biology of its members (Middlefart,

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2002). Phylogenetic evidence for a sister relationship between Archiheterodonta and the remainder of Heterodonta (Euheterodonta) is commonly recovered (Campbell, 2000; Park & Ó Foighil, 2000; Giribet & Wheeler, 2002;

Dryer et al., 2003; Giribet & Distel, 2003; Taylor & Glover, 2006; Harper et al., 2006; Taylor et al., 2007, 2009), but a sister group to Palaeoheterodonta has also been suggested (Sharma et al., 2012). The relationships within



FIGS. 1–3. Shells of *Carditamera* spp. FIG. 1: *Carditamera bajaensis*, sp. nov. (MCZ DNA106146_1) collected in La Paz, Baja California Sur, Mexico; FIG. 2: *Carditamera affinis* (G. B. Sowerby I, 1833) syntype (NHMUK) from the Golfo de Nicoya; FIG. 3: *Cardita californica* Deshayes, 1854, syntype (NHMUK) from the Golfo de California.

Archiheterodonta remain however unresolved (Giribet, 2008) but its monophyly is further supported by several morphological character systems, such as sperm ultrastructure (Healy, 1995). Hemoglobin has also been reported in several members of this clade (e.g., Taylor et al., 2005; Terwilliger & Terwilliger, 1985). Within Carditidae, extracellular hemoglobin has been reported in the blood of *Carditamera affinis* (G. B. Sowerby I, 1833).

The genus *Carditamera* Conrad, 1838, originally described from fossil specimens, can be distinguished from other members of Carditidae by an equivalve, oblong shell as well as hinge morphology, in which both strong cardinals and well-developed laterals are present, and is known to the Eocene (Conrad, 1838). As part of an ongoing systematic revision of Carditidae (VLG, work in progress) analysis of the phylogenetic breadth of this group was undertaken. Specifically, in examining species delimitations within Carditidae, molecular information elucidated species-level divergences within *Carditamera*, and thus, we report data that support the discovery of a new species.

Carditamera affinis was originally described from the Golfo de Nicoya, Costa Rica, but its distribution is said to extend north through Baja California and as far south as Peru (Huber, 2010). The morphological variability ascribed to *Carditamera affinis* is uncharacteristic of other species in the genus and was therefore reex-

amined for the possibility of additional species. Here, a new species of Carditidae belonging to the genus *Carditamera* is described from specimens previously considered *Carditamera affinis* (Figs. 1, 5, 13, 14).

MATERIAL AND METHODS

Abbreviations

Specimens are housed in the following institutions:

- | | |
|-------|---|
| MCZ | Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, U.S.A. |
| NHMUK | The Natural History Museum, London, England, U.K. |

Identification

Carditamera includes six species, but only *C. affinis* (Fig. 2) and *C. radiata* (G. B. Sowerby I, 1833) (Fig. 4) are known from tropical western Mexico. *Carditamera affinis* overlaps in distribution range with the new species reported here. *Carditamera affinis* can be distinguished by very thin-to-broad radial ribs, with scales or small spines on the posterior ends of the larger ribs. *Carditamera radiata* is not known from the Golfo de California, however it does



FIG. 4. *Carditamera radiata* (G. B. Sowerby I, 1833), syntype (NHMUK) collected in Salango, western Colombia and Panama from muddy sand from 11 to 18 m depth.

TABLE 1. List of MCZ accession numbers, collecting localities, and GenBank accession numbers for specimens used in molecular analysis.

MCZ Accession No.	Collection Site	GenBank Accession No.		
		ITS 2	16S rRNA	CYT B
DNA103800_1	Golfo de California, Puerto Peñasco, Bahía la Choya, Sonora, Mexico, G. Giribet, T. Hardy, M. K. Nishigushi 27.III.2003 [31°20'37"N, 113°38'38"W]	–	–	JX230961
DNA103800_2	"	JX230972	JX230955	JX230962
DNA106146_1	Golfo de California, Bahía Balandra, La Paz, Baja California Sur, Mexico, V. González & G. Kawauchi, 28.XII.2009 [24°19.019'N, 10°19.27'W]	–	–	JX230963
DNA106146_2	"	–	–	JX230964
DNA106146_3	"	–	–	JX230965
DNA106146_4	"	JX230973	JX230956	JX230966
DNA106146_6	"	JX230974	JX230957	JX230967
DNA106146_7	"	JX230975	JX230958	JX230968
DNA106147_1	"	JX230976	JX230959	JX230969
DNA106147_2	"	JX230977	JX230960	JX230970
DNA106147_3	"	–	–	JX230971

overlap with described distribution of *C. affinis* (Coan & Valentich-Scott, 2012). *Carditamera radiata* has smooth broad ribs, a short anterior end, a strongly tapering posterior end and has a maximal size of 56 mm. Huber (2010: 252) illustrated the new species under the name *Carditamera affinis*; Coan & Valentich-Scott (2012) used a illustrations of the new species for both *C. affinis* and *C. radiata*.

Anatomy

All specimens were preserved in ~ 96% ethanol for molecular work, as they originally were thought to be *Carditamera affinis*. No specimens suitable for histological study are thus available (Table 1).

Molecular Analysis

A total of 11 specimens were used in the molecular analysis (Table 1): nine *Carditamera* specimens collected in February 2009 from Bahía Balandra (24°19.019'N, 110°19.27'W) near La Paz, Baja California Sur (six from a sandy bottom substratum and three bysally attached to rocks); and two specimens collected in March 2003 from Bahía la Choya

(31°20'37"N, 113°38'38"W), Puerto Peñasco, Sonora, Golfo de California, found in the intertidal zone, buried in sand. Specimens and subsequent DNA extractions were retained as vouchers and are deposited in the Museum of Comparative Zoology, Department of Invertebrate Zoology DNA collection.

Total DNA was extracted from a small tissue sample from the mantle or foot using the DNeasy Tissue Kit (Qiagen) and the protocol provided by the manufacturer. The purified DNA was used as a template for PCR amplification of fragments of two mitochondrial genes (16S rRNA, and cytochrome *b*) and one nuclear gene (internal transcribed spacer region 2; ITS-2). The 16S rRNA gene was amplified and sequenced using primer pair 16Sa – 16Sb (Xiong & Kocher, 1991; Edgecombe et al., 2002). ITS-2 was amplified and sequenced using primer pair UCYTB144F and UCYTB272R (Merritt et al., 1998). The cytochrome *b* (CYT B) gene was amplified and sequenced using primer pair COBF and COBR (Passamonti, 2007).

PCR amplifications (25 µl) were conducted using 1 µL of the template DNA, 1 µL of each [100 µM] primer, 2.5 µL of EconoTaq 10X PCR buffer containing 15 mM MgCl₂ (Lucigen), 0.25

TABLE 2. List of specimens examined for morphological comparisons, including MCZ accession numbers, collecting localities, species, and number of specimens.

MCZ Accession No.	Collection Site	Species	No. of Specimens
DNA103800	Golfo de California, Puerto Peñasco, Bahía la Choya, Sonora, Mexico, G. Giribet, T. Hardy, M. K. Nishigushi 27.III.2003 [31°20'37"N, 113°38'38"W]	<i>C. bajaensis</i>	2
DNA106146	Golfo de California, Bahía Balandra, La Paz, Baja California Sur, Mexico, V. González & G. Kawauchi, 28.XII.2009 [24°19.019'N, 110°19.27'W]	<i>C. bajaensis</i>	7
DNA106147	Golfo de California, Bahía Balandra, La Paz, Baja California Sur, Mexico, V. González & G. Kawauchi, 28.XII.2009 [24°19.019'N, 110°19.27'W]	<i>C. affinis</i>	3
96	Golfo de California, N. W. Lermond Collection Acc. 665, C. R. Orcutt, collection date unknown	<i>C. affinis</i>	1
1300	Mazatlán, Mexico, W. J. Eyerdam, 14.XIII.1938	<i>C. affinis</i>	3
21674	Panama, E. R. Mayo, collection date unknown	<i>C. affinis</i>	4
29602	Collection locality unknown, Cal. Geol. Survey, collection date unknown	<i>C. affinis</i>	2
45084	Ciudad de Panamá, Panama, J. Zetek, collection date unknown	<i>C. affinis</i>	2
68798	Guaymas, Sonora, Mexico, C. R. Orcutt, collection date unknown	<i>C. bajaensis</i>	1
79238	San Felipe, Baja California, Mexico, J. M. Reed, 12.II.1928	<i>C. bajaensis</i>	3
100077	Ciudad de Panamá, Panama, J. Zetek, collection date unknown	<i>C. affinis</i>	3
110492	La Libertad, Sonora, Mexico, H. N. Lowe. II.1935	<i>C. bajaensis</i>	3
140875	Guaymas, Sonora, Mexico, H. R. Turner, 11.I.1942	<i>C. bajaensis</i>	2
148839	Bahía de San Carlos, Sonora, Mexico, F. Baker & L. G. Hertlein, 1921	<i>C. bajaensis</i>	10
148839	Bahía de San Carlos, Sonora, Mexico, F. Baker & L. G. Hertlein, 1921	<i>C. affinis</i>	2
174438	Puerto Peñasco, Sonora, Mexico, R. C. Beck, 30.IX.1948	<i>C. bajaensis</i>	2
176271	West Coast of Panama, C. M. Dumbauld, collection date unknown	<i>C. affinis</i>	1
198526	San Felipe, Baja California, Mexico, J. E. Fitch, 2.IV.1953	<i>C. bajaensis</i>	2
215636	Guaymas, Sonora, Mexico, C. Field, 1957	<i>C. bajaensis</i>	2
221093	Puertecitos, Baja California, Mexico, E. P. Chace. 11.II.1925	<i>C. bajaensis</i>	1
233102	Sandy Cove, Lagoon, Guayamas, Sonora, Mexico, J. W. R. & A. H. R., II.1940	<i>C. affinis</i>	4
245090	SW side of Bahía de las Ánimas, Gulf of California, Mexico, R. H. Parker, 1.IV.1959 [28°55'N, 113°31'W]	<i>C. bajaensis</i>	2
245125	Sargento (Sargent's Point), Sonora, Mexico, W. Emerson, III–IV.1962	<i>C. bajaensis</i>	2
263736	Venado, Panama, T. Dranga, XII. 1938	<i>C. affinis</i>	1
302867_DRY	El Requesón, 17 mi S of Mulegé, Baja California Sur, Mexico, S. P. & H. H. Kool, XII.1992	<i>C. bajaensis</i>	6
302867_WET	El Requesón, 17 mi S of Mulegé, Baja California Sur, Mexico, S. P. & H. H. Kool, XII.1992	<i>C. bajaensis</i>	11
302932	Small Bay, 15 mi S of Mulegé, Baja California Sur, Mexico, S. P. & H. H. Kool, 31.XII.1992	<i>C. affinis</i>	2
339587	San Felipe, Baja California, Mexico, J. Q. Burch, III.1938	<i>C. bajaensis</i>	2
339589	Guaymas, Sonora, Mexico, J.Q. Burch, 1947–1948	<i>C. bajaensis</i>	4
339589	Guaymas, Sonora, Mexico, J. Q. Burch, 1947–1948	<i>C. affinis</i>	11

(continues)

(continued)

MCZ Accession No.	Collection Site	Species	No. of Specimens
339592	Guaymas, Sonora, Mexico, J. Q. Burch, I.1948	<i>C. bajaensis</i>	3
339593	Bahía la Choya, Puerto Peñasco, Sonora, Mexico, T. & B. Burch, 25.XII.1966	<i>C. bajaensis</i>	1
339594	Guaymas, Sonora, Mexico, Mrs. H. R. Turver, II.1940	<i>C. bajaensis</i>	1
339595	Mazatlán, Mexico, J. Q. & R. Burch, XII.1960	<i>C. affinis</i>	4
339596	Mazatlán, Sinaloa, Mexico, collector and collection date unknown	<i>C. affinis</i>	4
339605	Carrizal Bay, Colima, Manzanillo, Mexico, L. & C. Shy, 1973–1974	<i>C. affinis</i>	1
339607	San Carlos, Guaymas, Sonora, Mexico, B. L. Burch, 6.I.1963	<i>C. affinis</i>	1
339608	San Carlos, Guaymas, Sonora, Mexico, B. L. Burch, collection date unknown	<i>C. affinis</i>	2
339609	San Carlos, Guaymas, Sonora, Mexico, J. Q. Burch, 3.XIII.1962	<i>C. affinis</i>	1
339610	Bahía la Choya, Puerto Peñasco, Sonora, Mexico, B. L. Burch, I.1967 [31°21'N, 113°41.2'W]	<i>C. affinis</i>	1

μL of dNTP's 100 mM, and 1.25 U of EconoTaq DNA polymerase (Lucigen). The PCR reactions were carried out using an Eppendorf Mastercycler epgradient thermal cycler. The thermal cycle program consisted of an initial denaturation at 95°C for 2 min, followed by 36 cycles of denaturation step at 95°C (45 s), annealing at 43–48°C (CYT B) or 48–53°C (16S rRNA and ITS 2) (1 min) and elongation at 72°C (90 s). A final elongation step at 72°C (4 min) and a rapid thermal ramp for 4°C were applied to finalize the process.

The double-stranded PCR products were visualized by agarose gel electrophoresis (1.5% agarose), cleaned with 2 μl of diluted (1:3) ExoSAP-IT (USB Corp., Cleveland, Ohio, U.S.A.) in a volume of 22 μL PCR product and performed at 37°C for 30 min followed by enzyme inactivation at 80°C for 15 min. Sequencing reactions were performed in a 10-μL reaction volume using 3.2 μL of primer (1 mM), 1 μL of ABI BigDye™ Terminator v. 3.0 (Applied Biosystems), 0.5 μL of BigDye 5 Sequencing Buffer (Applied Biosystems) and 3.3 μL of cleaned PCR product. The sequencing reaction, performed by using the thermal cycler described above, involved an initial denaturation step for 3 min at 95°C, 25 cycles (95°C for 10 s, 50°C for 5 s and 60°C for 4 min) and a rapid thermal ramp to 48°C. The BigDye-labeled PCR products were cleaned using Performa DTR Plates (Edge Biosystems, Gaithersburg, Maryland, U.S.A.).

The chromatograms were visualized, edited and assembled using Sequencher™ (Gene Codes Corporation, 1991–2011). Amplification primers were cropped and discarded from the edited sequences.

Multiple sequence alignment of molecular data (Table 1) was performed using MAFFT v. 6 using the default strategy (Kato et al., 2008) for each individual gene region. Haplotype networks were inferred using the statistical parsimony procedure implemented in the program TCS v. 1.21 (Clement et al., 2000), under default settings and assumptions (at 95% confidence interval), with indels treated as a fifth state.

Morphometric Analysis

Three morphological variables were measured following Soares et al. (1998): shell length (anterior-posterior), height (ventro-dorsal), and width (left-right) to 0.01 mm with Mitutoyo Absolute™ digital read calipers. Only specimens with complete and intact valves were measured; all single valve specimens were excluded (Table 2). Variance ratio tests ($p = 0.7565$), Shapiro-Wilk W test for normal data ($W/H, p = 0.39339$; $H/L, p = 0.13913$), and one-way ANOVA on morphological comparisons between width vs. height (W/H) and height vs. length (H/L) were performed in StataMP 12.0 (StataCorp, 1985–2011).

RESULTS

Molecular Analyses

Specimens collected in two contrasting habitats (sandy and rocky substrata) from two locations (Puerto Peñasco and La Paz) show habitat-specific morphological variation

(Figs. 1, 5–8) and comprise two genetically distinct groups. Based on descriptions by G. B. Sowerby I (1833), specimens from La Paz (byssally attached to rocks) have been identified as *Carditamera affinis*. Morphologically, the specimens found in sandy sediments at both locations are narrower and lack spines on the posterior ribs.



FIGS. 5–8. Representative morphologies of alcohol-preserved specimens used in the molecular analysis. FIG. 5: *Carditamera bajaensis*, sp. nov. (MCZ DNA103800_1) collected near Puerto Peñasco in exposed sand during low tide; FIGS. 6–8: *Carditamera affinis* specimens (MCZ DNA106147_1–3) collected off the coast of La Paz in and under rocks.

TABLE 3. List of *Carditamera bajaensis*, sp. nov. MCZ accession numbers, collecting localities, and number of specimens examined for species description.

MCZ Accession No.	Collection Site	No. of Specimens
79238	San Felipe, Baja California, Mexico, J. M. Reed, 12.II.1928	3
110492	La Libertad, Sonora, Mexico, H. N. Lowe, II.1935	3
140365	Golfo de California, San Juan, Baja California Sur, collector and collection date unknown	31
140875	Guaymas, Sonora, Mexico, H. R. Turner, 11.I.1942	2
148839	San Carlos Bay, Sonora, Mexico, F. Baker & L. G. Hertlein, 1921	12
174438	Puerto Peñasco, Sonora, Mexico, R. C. Beck, 30.IX.1948	2
198526	San Felipe, Baja California, Mexico, J. E. Fitch, 2.IV.1953	2
221093	Puertecitos, Baja California, Mexico, E. P. Chace, 11.II.1925	1
245090	SW side of Las Animas Bay, Gulf of California, Mexico, R. H. Parker, 1.IV.1959 [28°55'N, 113°31'W]	2
245125	Sargento (Sargent's Point), Sonora, Mexico, W.k. Emerson, III-IV.1962	2
254091	SW side of Las Animas Bay, Gulf of California, Mexico, R. H. Parker, 1.IV.1959 [28°55'N, 113°31'W]	2
302867_DRY	El Requeson, 17 mi S of Mulege, Baja California, Mexico, S. P. Kool & H. H., XII.1992	6
302867_WET	El Requeson, 17 mi S of Mulege, Baja California, Mexico, S. P. & H. H. Kool, XII.1992	11
339587	San Felipe, Baja California Norte, Mexico, J. Q. Burch, III.1938	3
339589	Guaymas, Sonora, Mexico, J. Q. Burch, 1947–1948	4
339592	Guaymas, Sonora, Mexico, J. Q. Burch, I.1948	3
339593	Cholla Bay, Puerto Peñasco, Sonora, Mexico, T. & B. L. Burch, 25.XII.1966	1
339594	Guaymas, Sonora, Mexico, Mrs. H. R. Turver, II.1940	2

Haplotype analysis shows disconnected networks for all loci (ITS-2, 16S, CYT B) (Figs. 9–11). All three gene regions show a clear distinction between the haplotypes collected in the sandy substrata (Puerto Peñasco [sand] + La Paz [sand]) versus haplotypes collected on rocky substrata [La Paz (rocks)]. Small byssally attached individuals have also been observed intertidally in rocks at La Paz, but the specimens were not collected (G. G., March 2003). After relaxation of the 95% confidence interval, 69 mutational steps are required to connect the sandy versus rocky haplotypes, while only two steps separate the sandy substratum haplotypes for the ITS-2 gene region (Fig. 9). CYT B and 16S rRNA gene information require 41 and 29 mutational steps to connect the haplotypes, respectively (Figs. 10, 11). No haplotypes are shared between rocky (*C. affinis*) and sandy (*C. bajaensis*, sp. nov.) forms for any marker.

Morphometric Comparisons

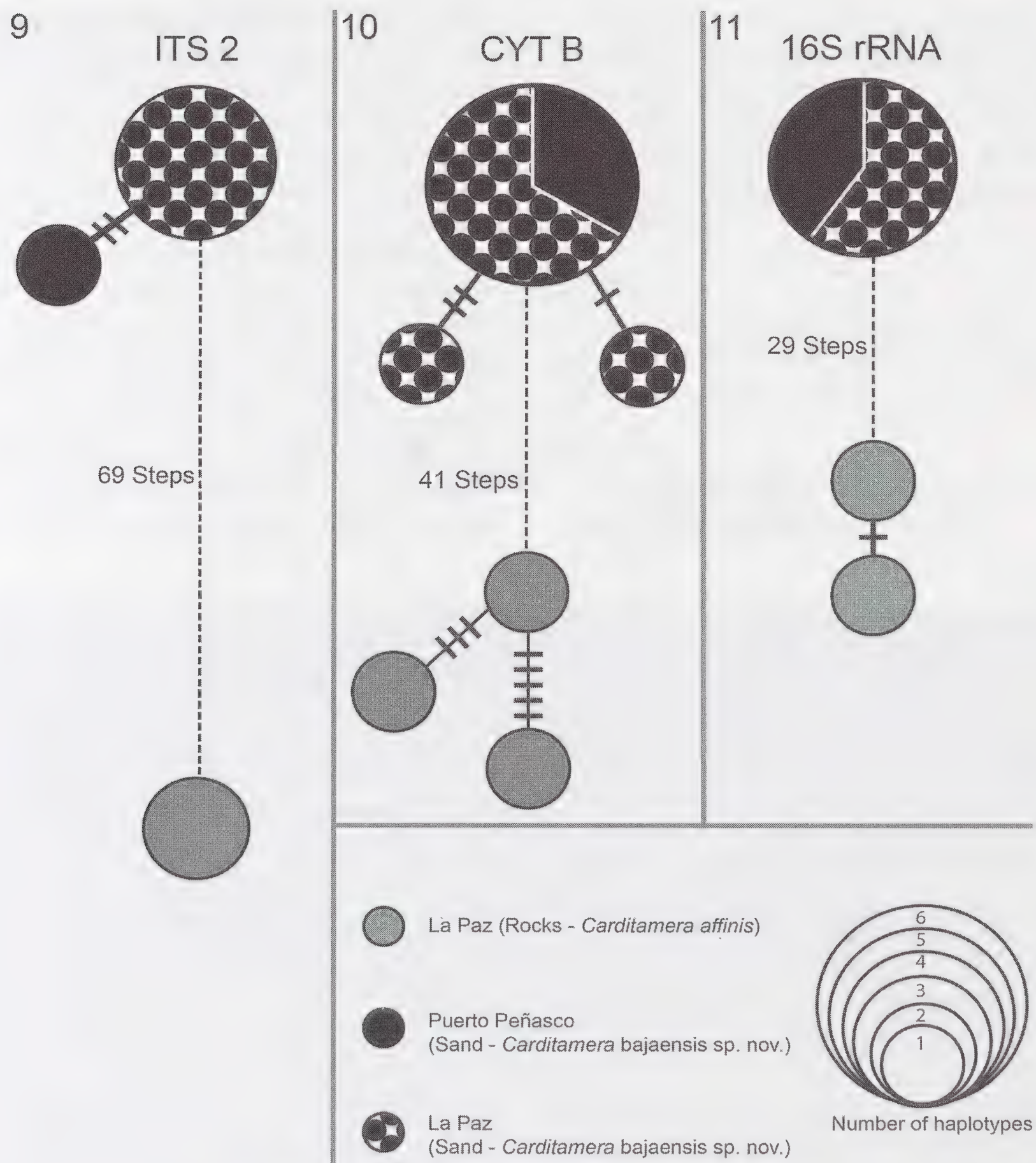
Morphological comparisons between width vs. height (*W/H*) and height vs. length (*H/L*) revealed a significant difference in shell shape between *C. affinis* and *C. bajaensis*, sp. nov., which further corroborates morphological distinctiveness (One-way ANOVA, *p* = 0.0419). *Carditamera affinis* specimens were wider and rounder, whereas *C. bajaensis* specimens were thinner and flatter (Fig. 12).

SYSTEMATICS

Taxonomy

Carditidae Férussac, 1822

Type genus: *Cardita* Bruguière, 1792
Type species: *C. variegata* Bruguière, 1792, by subsequent designation of Gray, 1847



FIGS. 9–11. TCS networks. FIG. 9: Network based on ITS-2 data; FIG. 10: Network based on CYT B data; FIG. 11: Network based on 16S rRNA data. Representative haplotypes from the three localities are indicated above. The size of the circle is proportional to the number of represented haplotypes. Solid lines connect haplotypes with a single step (inferred intermediate haplotypes are indicated by a hash mark); a dashed line represents relaxation of 95% confidence limit.

Carditamera Conrad, 1838
Byssomera Olsson, 1961
Lazaria Gray, 1854

Type species: *Carditamera arata* (Conrad, 1832) by original designation. Miocene, eastern North America.

Carditamera bajaensis, sp. nov.
 Figs. 1, 5, 13, 14

Types

Holotype: (MCZ DNA106146_1) (41 mm long x 18 mm high x 13 mm wide) from Bahía Balan-

dra (24°19.019'N, 110°19.27'W), La Paz, Baja California Sur, Mexico, depth 1 m, collected 28 February 2009 by V. L. González & G. Y. Kawauchi (on sandy bottom substratum).

Paratypes: Six specimens (MCZ DNA 106146_2-7), same collecting data as holotype; two specimens (MCZ DNA103800_1-2) (78 mm long x 32 mm high x 26 mm wide) collected from Bahía la Choya (31°20'37"N, 113°38'38"W), Golfo de California, Puerto Peñasco, Sonora, Mexico, depth 1 m, collected 27 March 2003 by G. Giribet (in exposed sand); and two specimens (MCZ 245090), collected from the southwest side of Bahía de las Ánimas (28°55'N, 113°31'W), Golfo

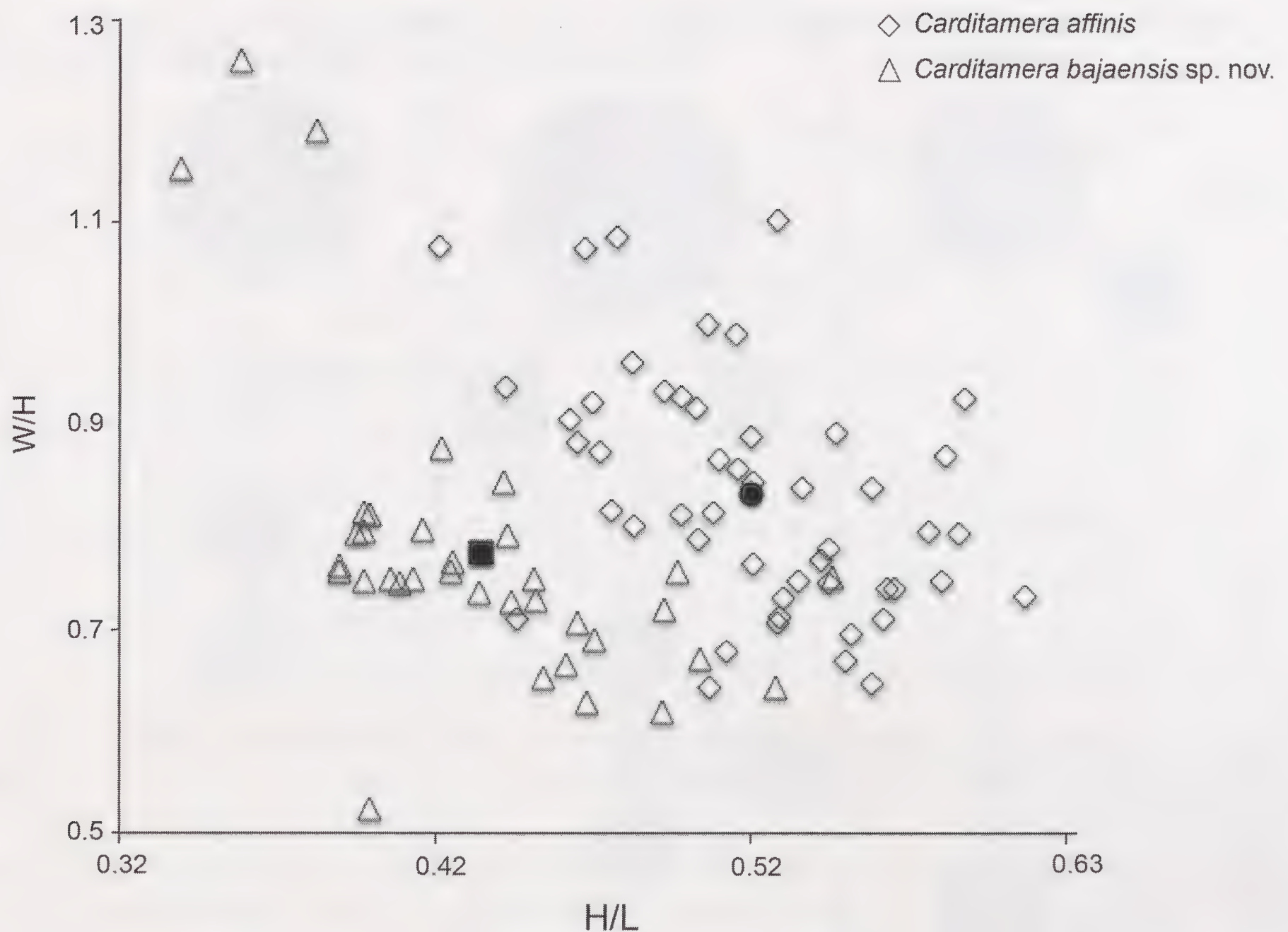


FIG. 12. Morphometric comparisons of *C. bajaensis*, sp. nov. (Triangles) and *C. affinis* (Diamonds). Measurements of W/H plotted against H/L ; Means of W/H and H/L for *C. bajaensis*, sp. nov. (filled square) and *C. affinis* (filled circle) are 0.78 and 0.43; and 0.84 and 0.51, respectively.

de California, Baja California Sur, Mexico, collected 1 April 1959 by R. H. Parker.

Additional Material Studied

Eighteen lots, both dry shell collections ($n = 17$ lots) and one alcohol-preserved lot (MCZ 302867), totaling 92 specimens housed in the Museum of Comparative Zoology, Department of Malacology (Table 3).

Material Examined for Comparison

Carditamera affinis (G. B. Sowerby I, 1833): three syntypes (NHMUK) collected in the Bahía de Montejó and Golfo de Nicoya, Costa Rica. *Carditamera* cf. *affinis* (formerly *Cardita californica* Deshayes, 1854): 3 syntypes (NHMUK) from the collection of Hugh Cumming, collected in the Golfo de California.

Etymology

The specific epithet refers to the state where the type locality of the holotype specimen is found, Baja California Sur, Mexico.

Diagnosis

Moderately sized *Carditamera*, 41 to 85 mm long in measured specimens, narrow, ribbed, thick-shelled bivalve. Shell elongate, quadrangular, twice as long as high, with approximately 15 smooth flat prominent ribs of equal distinctness over the whole shell; longer posteriorly. Color white, banded with brown; lacking spinose posterior ribs like those of *C. affinis*. Pilose thick periostracum; brown coloration.

Description

Posteriorly elongate shell with hinge line parallel to straightened ventral margin (Fig. 1). Interior color whitish with brown coloration, non-nacreous. Shell equivalve and inequilateral. Umbones anterior, prosogyrate. Lunule small and deep; escutcheon weakly distinct. Shell margin crenulate, both in the interior and exterior of the valves, following the radial ribs. Pilose periostracum only apparent around margins (Fig. 13).

Hinge plate wide. Hinge structure with strong lateral teeth, 2 right anterior laterals (la1 and la2) and one right anterior cardinal (3a) and two corresponding left anterior cardinal teeth (2a and 2b) (Fig. 14). External ligament present, opisthodetic, parivincular. Pallial line complete, without pallial sinus. Papillate posterior mantle margin present, with the posterior mantle folds fused to form posterior excurrent aperture; mantle margins not fused ventrally. Carinate foot pointed posteriorly. Byssal groove present and with byssus occasionally present in adult stage [2 of N = 8], yet rare. Anterior and posterior pedal retractor muscles present. Heteromyarian, with a slightly larger posterior adductor; the smaller anterior adductor at-

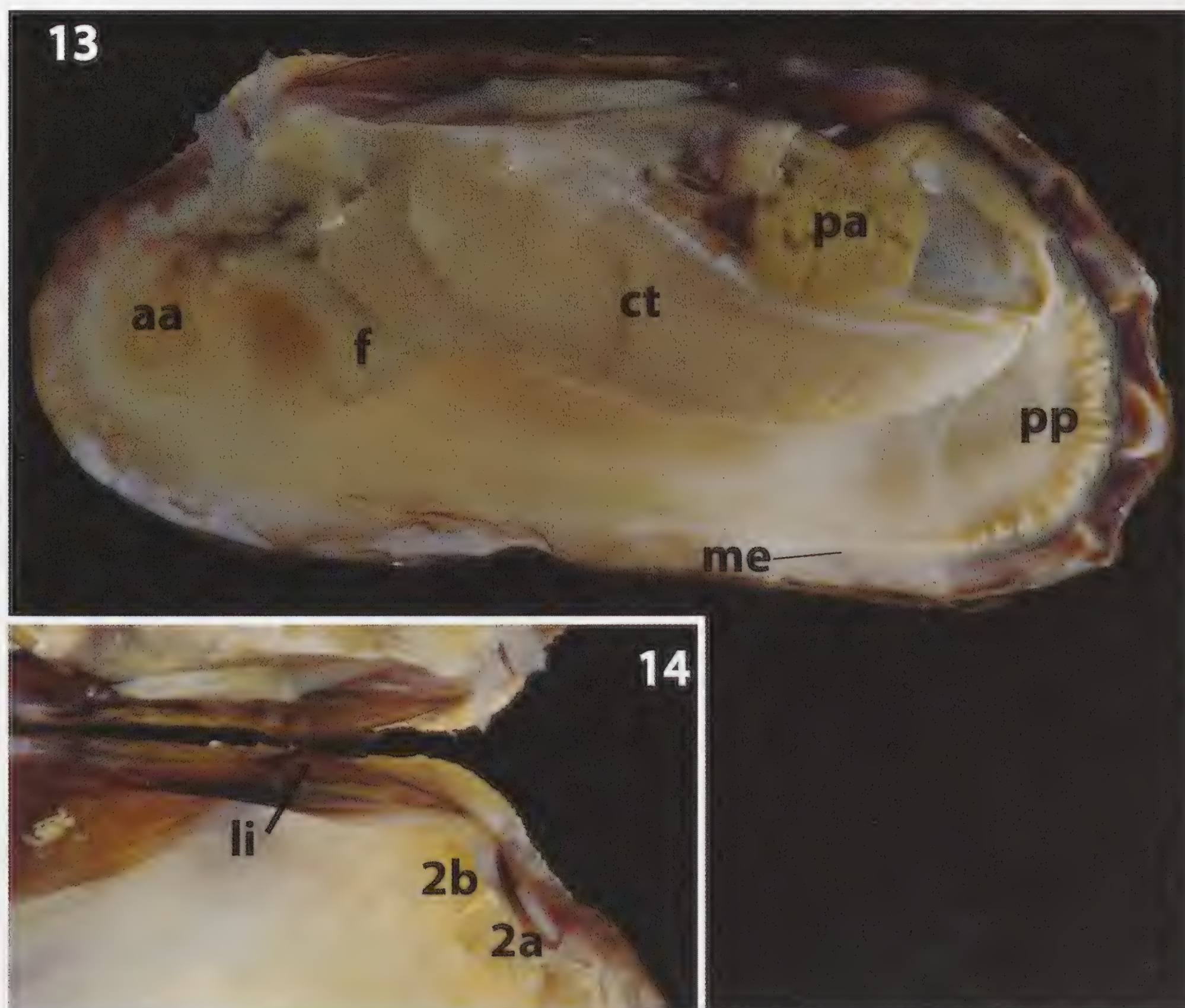
tached to an antero-ventral extension of the valves. Eulamellibranch ctenidia, homorhabdic, with outer demibranch slightly smaller than inner demibranch. Labial palps small, triangular in shape. Stomach structure not elucidated, with a spiral typhlosole. Midgut uncoiled.

Habitat

Semi-infaunal; specimens have been collected buried in exposed and intertidal sandy beach environments.

Distribution

Known from several collecting localities within the Golfo de California (Fig. 15).



FIGS. 13, 14. Alcohol-preserved specimen of *Carditamera bajaensis*, sp. nov. (MCZ DNA106146_1). FIG. 13: Animal with left mantle and valve removed; FIG. 14: Left valve showing hinge and cardinal teeth. Abbreviations: aa, anterior adductor muscle; me, mantle edge; ct, ctenidium; f, foot; li, ligament; pa, posterior adductor muscle; pp, posterior papillae; 2a, left anterior cardinal tooth; 2b, left middle cardinal tooth.

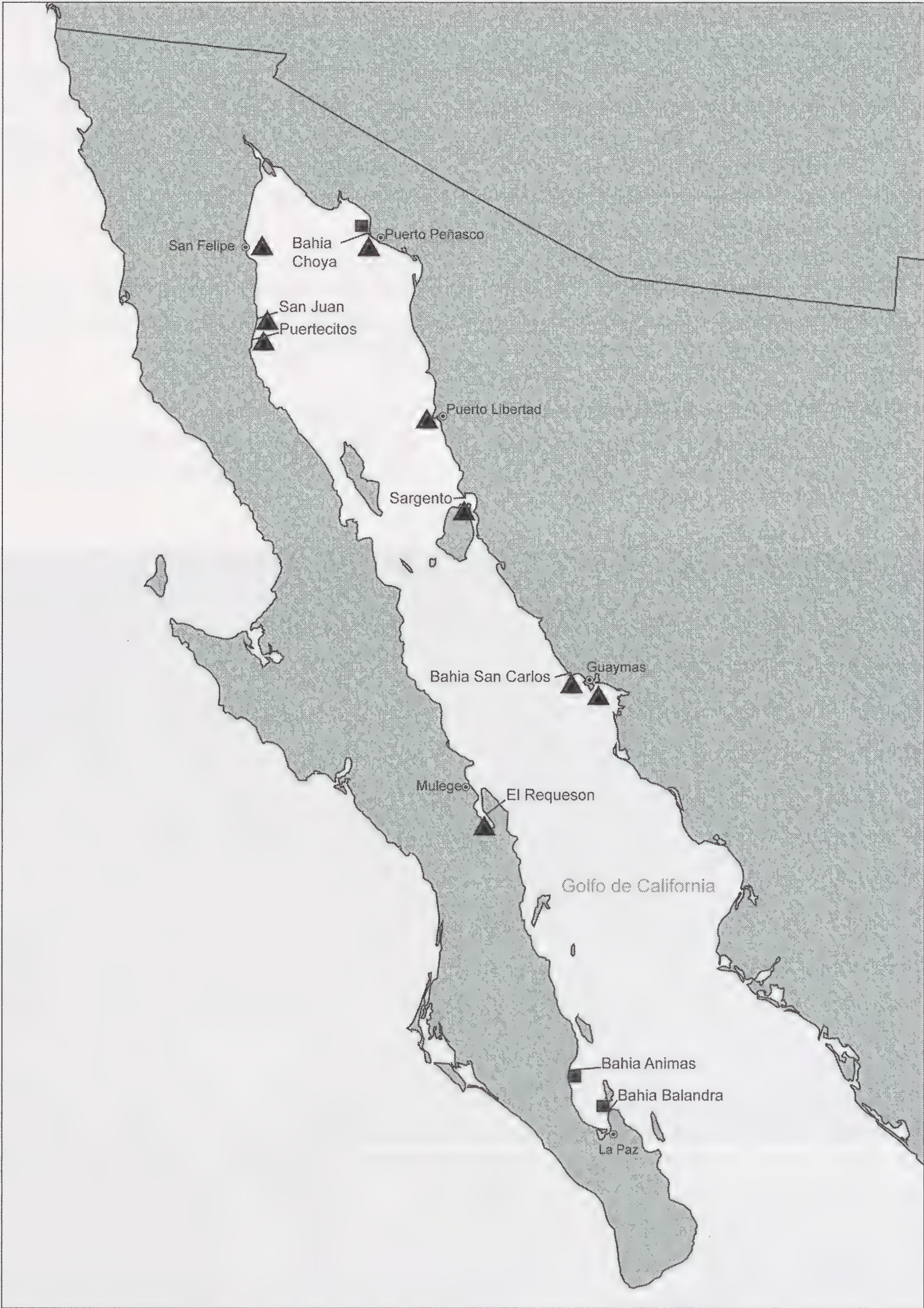


FIG. 15. Map of the Golfo de California showing known localities for *Carditamera bajaensis*, sp. nov. Exact collecting localities represented by a black square; approximate collecting localities indicated by a black triangle based on Table 3.

DISCUSSION

The three syntypes of *Carditamera affinis* (NHMUK) (one specimen pictured in Fig. 2) are large elongate shells, in which the length is about twice the height, and have 15 or more ribs present. Prominent posterior scales are present on the exterior of the shell and speci-

mens have coloration ranging from brownish white to brown. *Carditamera affinis* is more globose than *C. bajaensis*, and the shell is slightly broader than deep. Compared with *C. affinis*, *C. bajaensis* is more elongate, less globose, has a more linear ventral margin and lacks prominent large posterior scaly projections. *Carditamera affinis* is epifaunal, found

in crevices or under rocks, usually byssally attached to the substratum, sometimes exposed in the intertidal zone; ***Carditamera bajaensis*** is semi-infaunal and has been collected in exposed sandy beach environments at low tide. To evaluate the validity of ***C. bajaensis***, all five known synonyms of *C. affinis* – *Cardita californica* Deshayes, 1854; *Cardita incerta* Clessin, 1888; *Cardita petunculus* Reeve, 1843; *Cardita picta* Clessin, 1888; *Cardita volucris* Reeve, 1843 – were investigated and each original description was checked.

As indicated by Keen (1971: 107), *Carditamera* cf. *affinis* (formerly *Cardita californica* Deshayes, 1854) was described as a smoother northern subspecies of *Carditamera affinis* (one specimen pictured in Fig. 3). However, upon reexamination of the syntypes of *Cardita californica* (NHMUK) (Fig. 3), the specimens have globose elongate shells with distinct posterior scales, and are similar in shape and periostracum color to those of the *C. affinis* syntypes, unlike the smooth exterior and narrower width of ***C. bajaensis***, further indicating that *Cardita californica* and *C. affinis* are conspecific.

Clessin (1888) described two species that have subsequently been synonymized with *C. affinis*. Both *Cardita picta* Clessin, 1888, and *Cardita incerta* Clessin, 1888, were described as having wide rounded shells with a total of 19 radial ribs, differing from the narrow shell and 15 ribs of ***C. bajaensis***. Reeve (1843) also described two species that have been synonymized with *Carditamera affinis*: *Cardita petunculus* Reeve, 1843, is a large form from Madagascar that lacks banding and spotted coloration characteristic of ***C. bajaensis***, and *Cardita volucris* Reeve, 1843 (type locality unknown), which has an elongate, globose, and scaly shell, and appears to be a true synonymy of *C. affinis*.

Haplotypic networks and phylogenetic analyses have been recently used to elucidate cryptic species of marine invertebrates (e.g., Baker et al., 2007; Duran & Rützler, 2006; Kawauchi & Giribet, 2010), including molluscs (e.g., Marko & Moran, 2009; Kawauchi & Giribet, 2011; Zamborsky & Nishiguchi, 2011), as they have the power to compare the degree of within species and among species genetic diversity. The case of ***Carditamera bajaensis*** is clear, as all characters, from shell anatomy (little data are available for the internal anatomy of related *Carditamera* spp.), ecological niche, and haplotypes, support the reciprocal monophyly of the sampled populations. Furthermore,

the specimens here described as ***Carditamera bajaensis*** cannot be assigned to any of the previously described species now considered synonyms of *Carditamera affinis* or any other *Carditamera* described from this region. This, in addition to the molecular sequence data that distinguish the two life forms for every examined marker, but that unite populations of the infaunal species separated by about 800 km, confirms a distinct and new species, ***Carditamera bajaensis***. Ongoing revision of Carditidae, in concert with molecular tools, is anticipated to contribute significantly to the systematics and known diversity of constituent genera.

Evidence for cryptic speciation in the light of phenotypic plasticity has been investigated within several molluscan groups (Richter et al., 2008; Kawauchi & Giribet, 2011). Traditionally, taxonomy within Bivalvia has relied heavily on characters of shell morphology. However, reliance on shell morphological characters alone, which has a propensity in conchiferans for both cryptic speciation (e.g., Won et al., 2003b; Lee & Ó Foighil, 2004; Johnson et al., 2009; Lorion et al., 2010) and environmental plasticity (Yeap et al., 2001; Wulfschleger & Jokela, 2002; Baker et al., 2003, 2004; Hollander et al., 2006; Pfenninger et al., 2006; Lorion et al., 2010), can belie the estimation of diversity within these groups.

Convergence and parallelism are rampant within Bivalvia, not only with respect to morphology, but also mode of life (Alejandrino et al., 2011), and more integrated approaches are necessary to delimit species (Harper et al., 2000). This approach is exemplified by the study of Richter et al. (2008), in which morphological, in concert with genetic and ecological data, were used to describe a distinct new species of *Tridacna* in the Red Sea; previously, large phenotypic plasticity had mistakenly been ascribed to a single taxon.

Evolutionary plasticity of life habitat has played an important role in bivalves, both in response to environmental changes (Soares et al., 1998) and for driving diversification (Marko & Jackson, 2001). Environmental plasticity may have facilitated colonization of new habitats by *Carditamera*, followed by niche partitioning and ecological speciation (e.g., in habitats with varying substrata). The sympatric distributions of *C. affinis* and ***C. bajaensis***, and the segregation of their respective substrate type is suggestive of a role for evolutionary plasticity as a driver for the diversification within the genus in the Golfo de California. Testing this hypothesis utilizing morphological, ecological,

and population genetic data requires denser sampling of *Carditamera* than is currently available, and therefore remains an objective for future investigations.

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A HISTOLOGICAL STUDY OF OOGENESIS IN THE FRESHWATER MUSSEL *ANODONTA CYGNEA* (LINNAEUS, 1758) IN MIRA LAGOON, PORTUGAL

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ABSTRACT

Oogenesis of the freshwater mussel *Anodonta cygnea* was investigated through histological examination over a 12-month period. All specimens were hermaphrodites, having gonads made up of numerous separate female and male acini. Female acini included oogonia with oocytes at different stages of development. Immature oocytes were attached to the acinar wall by a stalk, while mature oocytes were free within the acinus lumen. Several male and female acini were connected to a single germinal duct filled with mature germ cells. A maturity gradation scale was developed, which incorporated the characteristics of the acini, germinal epithelium, lumen and connective tissue. Although oogenesis seems to occur throughout the year, this scale has shown that oogenesis peaks during the summer months.

Key words: Unionidae, *Anodonta*, acini, germinal duct, oogonia, oocytes, maturity gradation scale.

INTRODUCTION

The native freshwater mussels are among the most endangered species of Iberian freshwater fauna. Until a few years ago, these large unionids were abundant in Portuguese freshwater ecosystems. However, due to the overall degradation of the habitat, the species belonging to this group are experiencing great decline. Thus, it is imperative to establish practical conservation measures, such as the reintroduction of artificially cultured mussels. A relevant factor for the successful breeding of bivalve species lies in understanding their key biological processes, the most important of which are their reproductive features and cycle.

Bivalve molluscs are typically gonochoristic (Coe, 1943; Morton, 1991), although among freshwater bivalves there are some hermaphroditic species (van der Schalie, 1969, 1970; Heard, 1975; Morton, 1991; Araújo, 1995). Historically, studies on Unionoidea indicate that, depending on the species, hermaphroditism can be a rare or occasional event (van der Schalie, 1966; Heard, 1975; Smith, 1979; Dudgeon & Morton, 1983; Kat, 1983). In addition,

when hermaphroditic individuals do exist, they occur at low frequencies (Heard, 1979).

The reproductive cycle of *Anodonta cygnea* is similar to that of other freshwater mussels. There is evidence of summer gametogenesis, with spawning in the early autumn followed by glochidia brooding and subsequent release in winter (Galhano & Silva, 1983). Like most freshwater mussels, *A. cygnea* was considered gonochoristic (Galhano & Silva, 1983). However, there are studies reporting *A. cygnea* hermaphroditic populations (Bloomer, 1930), as well as sex-changing and self-fertilizing specimens (Ellis, 1978). Spermatogenesis has already been well documented by Rocha & Azevedo (1990). Since then, however, no recent research work has been carried out to study the reproductive cycle of this species, despite the numerous changes that may have occurred in its reproductive pattern and habitat (Lima, unpublished).

Regarded as the most reliable methodology to assess seasonal trends in reproduction (Seed & Suchanek, 1992; Barber, 1996), histological analysis is essential to study the events taking place during bivalve gonadal development.

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In spite of this, the stages of gonad development in freshwater mussels (Wang & Denson, 1995; Juhel et al., 2003), which are similar to those previously advanced for marine mussels and other invertebrates (Wilson & Simons, 1985; Villalba, 1995; Barber, 1996; Parker et al., 1999; Cronin et al., 2000), are usually established by means of subjective qualitative evaluation of the shape and nature of gonadal cells. When a subjective classification system is used, measurement accuracy necessarily relies on the researcher's skill and experience to correctly identify reproductive stages. In contrast, quantitative techniques remove much of the subjective error from data collection and also provide better opportunities for descriptive and comparative analysis (Buchanan, 2001). A quantitative methodology is therefore needed (Barber, 1996). Thus, this paper aims to describe, for the first time, the oogenesis of *A. cygnea* through qualitative and quantitative histological assessment of gonad morphology. Our study focused on the female germ cells due to their complexity, whereas variation in testis condition showed a clear and distinct pattern that can be easily classified by means of the current descriptive staging methods used for other mussel species. Since this species is at the brink of extinction in Portugal, this study is crucial to determine the species reproductive cycle according to which management plans for its conservation must be established. In order to be able to control the whole mussel life cycle, its reproductive biology and gonad development must first be understood.

MATERIAL AND METHODS

The population under analysis was located in Mira Lagoon, approximately 15 km from the city of Aveiro in northern Portugal (40°26'20"N). This location was chosen as a study site due to the presence of a relatively large and significantly dense population of natural mussels. *Anodonta cygnea* was sampled monthly from October 2004 to September 2005. Due to the endangered status of this species, it was necessary to preserve the natural populations and therefore the number of specimens used was kept low (six specimens per month).

Histology

Although inadvisable for endangered species, the most accurate method for sexing unionoids

involves histological examination. For each specimen, the soft body tissue was detached and removed from the shell using a scalpel. Sections of the dissected foot were immediately fixed in Bouin's solution (Panreac, Barcelona, Spain) for one week and stored in 70% ethanol for no longer than two months. Samples were then dehydrated in an ascending series of ethanol solutions, cleared in xylene (Merck, Darmstadt, Germany) and embedded in paraffin (Merck). Paraffin blocks were sectioned (5 µm thick) using a Leitz 1512 microtome, and the sections mounted on glass slides. After deparaffinization in xylene, and rehydration in a descending series of ethanol solutions, the sections were stained with haematoxylin (Merck) and eosin (Merck; Sheehan & Hrapchak, 1980). Finally, sections were mounted under a coverslip with DPX (BDH Laboratory Supplies, Poole, England). Observations were made with a Leitz Aristoplan compound microscope. Photographs of representative sections were taken with an Olympus digital camera model C5050Z (Olympus, Germany), and the images were then assembled and labelled with Photoshop 5.0 (Adobe Systems, San Jose, California, USA).

Staging of Gonads

After several approaches using the existing descriptive staging methods (Wilson & Simons, 1985; Wang & Denson, 1995; Villalba, 1995; Barber, 1996; Parker et al., 1999; Cronin et al., 2000; Juhel et al., 2003), we were not able to establish a regular pattern to assess the gonadal maturation in *A. cygnea*. Bearing in mind the subjectivity of these qualitative methods, a quantitative methodology was adopted to stage the development phase of the gonads. In order to do this, constant numbers ($n = 50$) of follicles were analysed in each of the 24 specimens and the presence of particular features were carefully quantified. The following characteristics were considered: regularity and continuity of the acini wall; continuity, thickness and cell types of the epithelium; lumen size and type of cells within; and finally, the cell type found in the connective tissue. The acini with regular and continuous walls were considered as more immature, as were those having continuous, thick germinal epithelia with a higher proportion of undifferentiated cells. The acini without lumen are characteristically less mature, with increasing lumen size representing a higher level of gonad development. Among

TABLE 1. Stage delimitation for each cellular characteristic of the female acini in *Anodonta cygnea*. *Stage 1 is the most immature, maturity increasing as the stage number rises.

		Stage 1*	Stage 2	Stage 3	Stage 4	Stage 5
Acini	Regular	37–42	32–36	27–31	22–26	17–21
	Irregular	8–13	14–18	19–23	24–28	29–33
	Continuous wall	42–50	31–41	22–32	13–23	5–14
	Discontinuous wall	0–8	9–17	18–26	27–35	36–45
Germinal Epithelium	Continuous	39–50	29–40	19–30	9–20	0–10
	Discontinuous	0–9	10–19	20–29	30–39	40–50
	Thick	41–50	31–40	21–30	11–20	0–10
	Thin + Thick	0–4	5–8	6–9	≤ 9	≤ 4
	Thin	0–12	0–12	13–29	20–34	35–50
	Undifferentiated	≥ 15	10–14	5–9	0–4	0–4
	Oogonia	< 5	5–7	5–7	8–14	0–7
	Oocytes	0–28	0–28	14–38	14–38	28–48
	Pedunculated oocytes	≤ 10	≤ 10	≤ 12	4–20	4–20
Lumen	Absent	> 20	10–20	10–20	0–9	0–9
	Small	13–21	13–21	20–28	15–26	5–14
	Medium	0–7	8–17	8–17	12–20	> 20
	Large	0–4	1–4	2–5	4–12	4–22
	Total Absent					
	Epithelium	0.8–1	0.6–0.8	0.4–0.6	0.2–0.4	0–0.2
	Mature Oocytes + Atretic	0–0.1	0.2–0.3	0.4–0.5	0.6–0.7	0.8–1
	Total Acini with Lumen					
	Empty	≥ 25	0.15–0.24	0.15–0.24	0–0.14	0–0.15
	Mature Oocytes	0.2–0.4	0.2–0.4	0.5–1	0.5–1	0–0.5
	Mature + Atretic	0–0.22	0.19–0.4	0.02–0.3	0.02–0.3	0.26–0.5
	Atretic	0–0.21	0–0.3	0–0.3	0–0.6	0–0.6
Connective Tissue		no Oocytes/ no Haemocytes	Oocytes	Oocytes + Haemocytes	Haemocytes	-

acini without lumen, those lacking germinal epithelia were considered to be in immature stages, whereas among acini with lumen, those with a higher proportion of mature and atretic oocytes were included in more mature stages. The numbers of female acini with or without the characteristics referred in Table 1 were counted, and the values obtained for each cellular feature were divided into numerical intervals and combined in order to develop a five-stage (1–5) maturity gradation scale (MGS). Stage 1 was defined as the least mature and included the intervals with higher numbers of immature

characteristics and with a lower proportion of maturation signs. On the other hand, stage 5 was assigned to specimens with higher numbers of more mature characteristics. To each acini feature and according to the classification key in Table 1, a MGS stage was attributed to each specimen. For example, a specimen with 40 acini of regular shape walls, or with 45 acini of continuous walls, would be assigned a MGS stage 1 rating. This step allowed for the study of individual features over time as well as for the determination of a cumulative index (Table 2). For each mussel, the MGS stages

TABLE 2. Cumulative stage delimitation for *Anodonta cygnea* specimens.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Cumulative Stages	16–20	21–25	26–30	31–35	≥ 36

attributed to each characteristic were added, each characteristic carrying the same relative weight. Final values were divided into intervals of similar amplitude to generate a five-stage cumulative index in which those of lower values were defined as stage 1. This index was used to classify each specimen so as to evaluate the global magnitude of seasonal changes.

Statistical Analysis

Seasonal differences were tested by means of the Kruskal-Wallis one-way ANOVA. The Mann-Whitney Rank Sum Test, with 95% confidence limits, was applied to compare the means whenever a significant difference was found (SigmaStat, Statistical software, SPSS, Inc).

RESULTS

The mussels analysed had a mean length of 12.7 ± 2.5 cm, with a maximum of 17 cm and minimum of 9.4 cm. Concerning weight, the mean value was 256.0 ± 90.5 g, ranging from 132.8 g to 401.5 g.

Qualitative Analysis

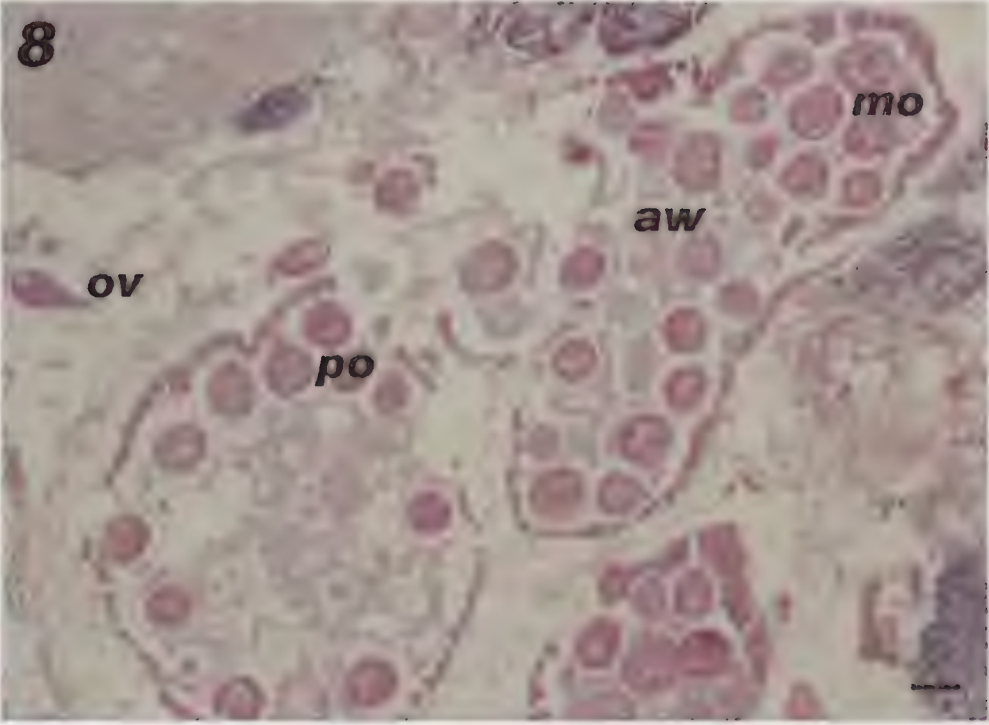
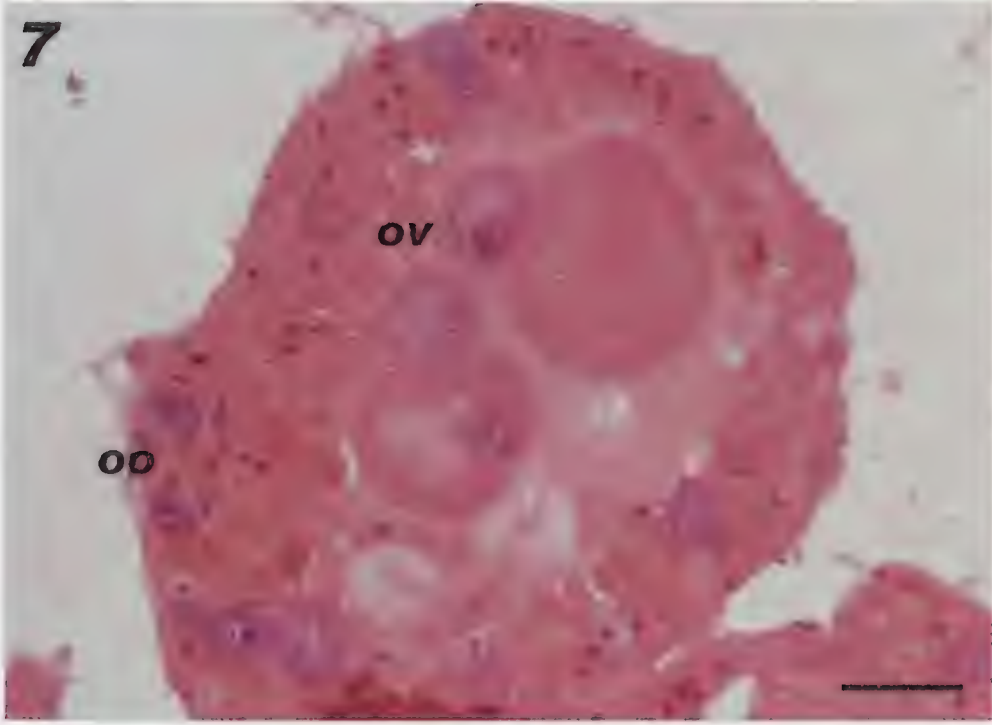
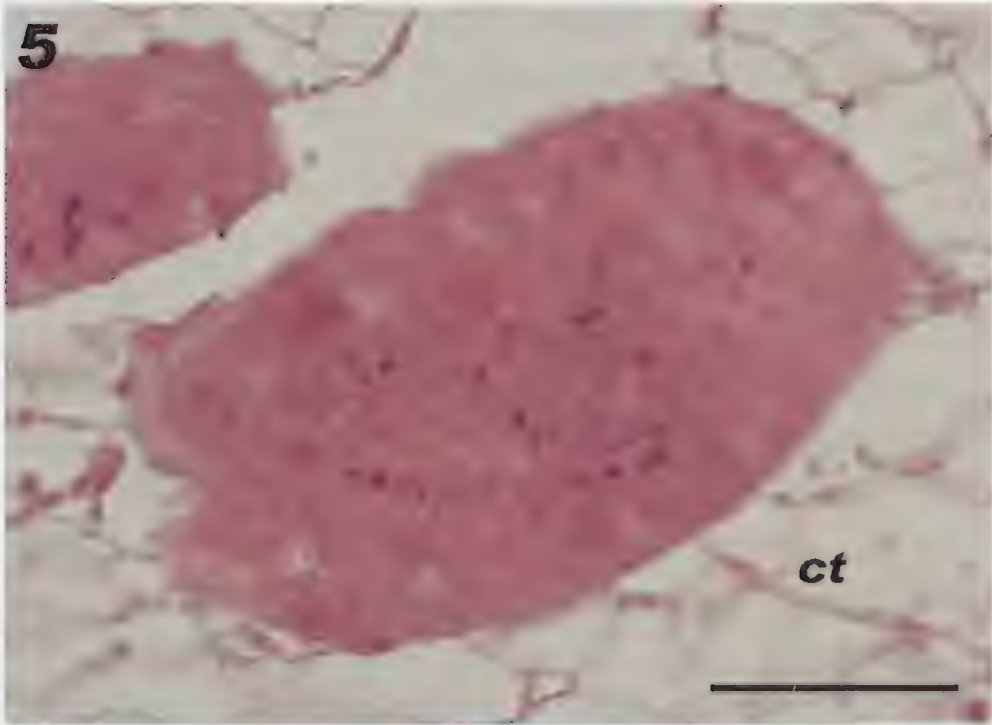
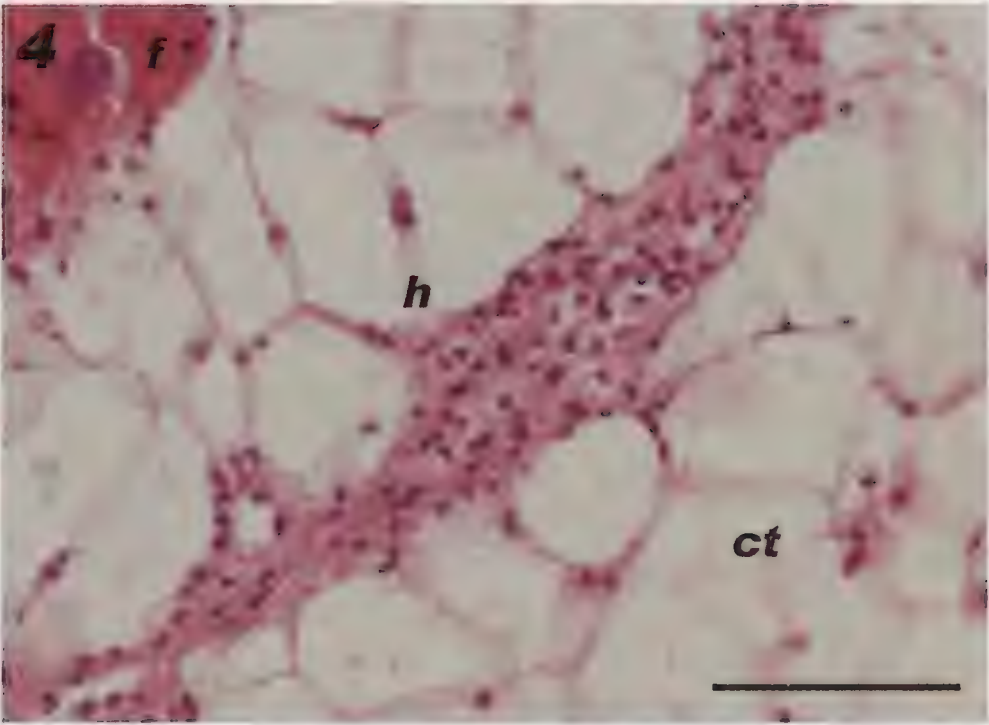
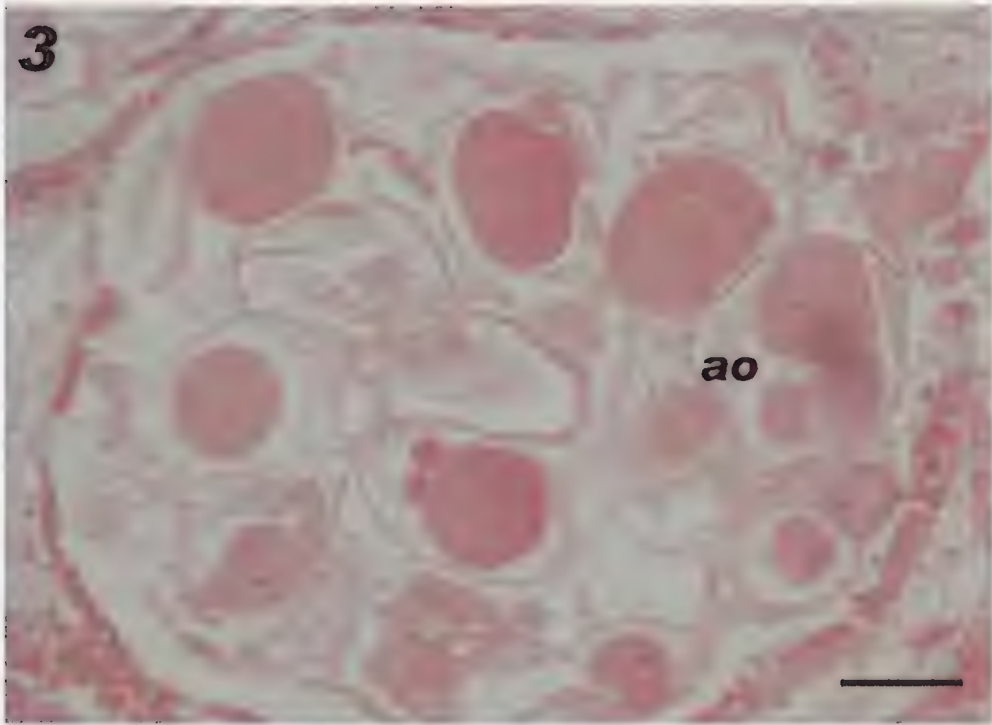
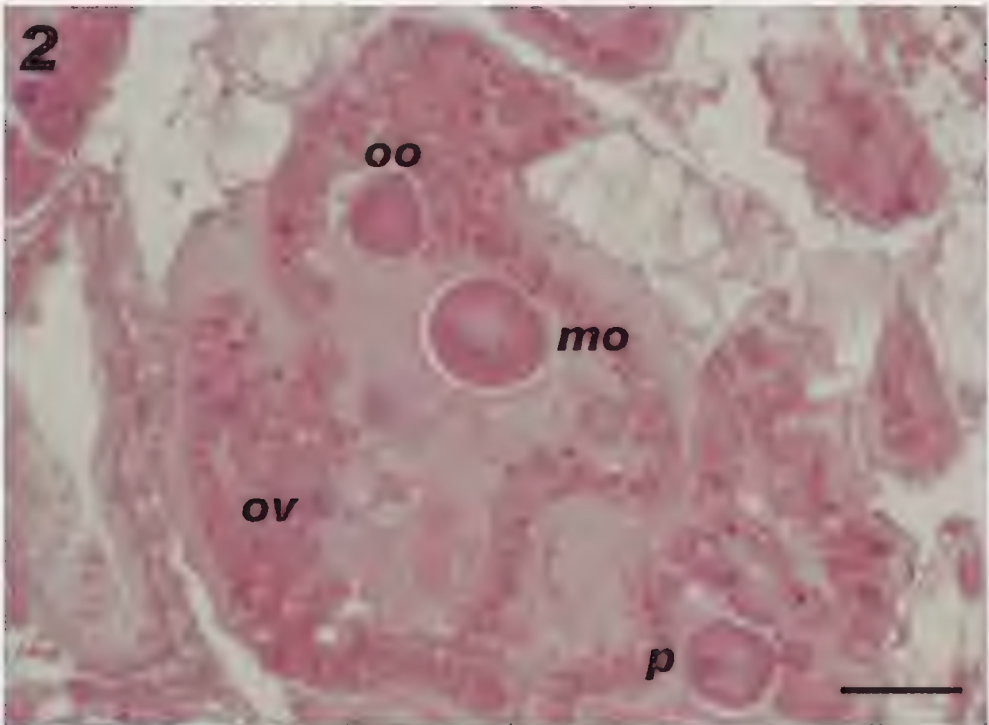
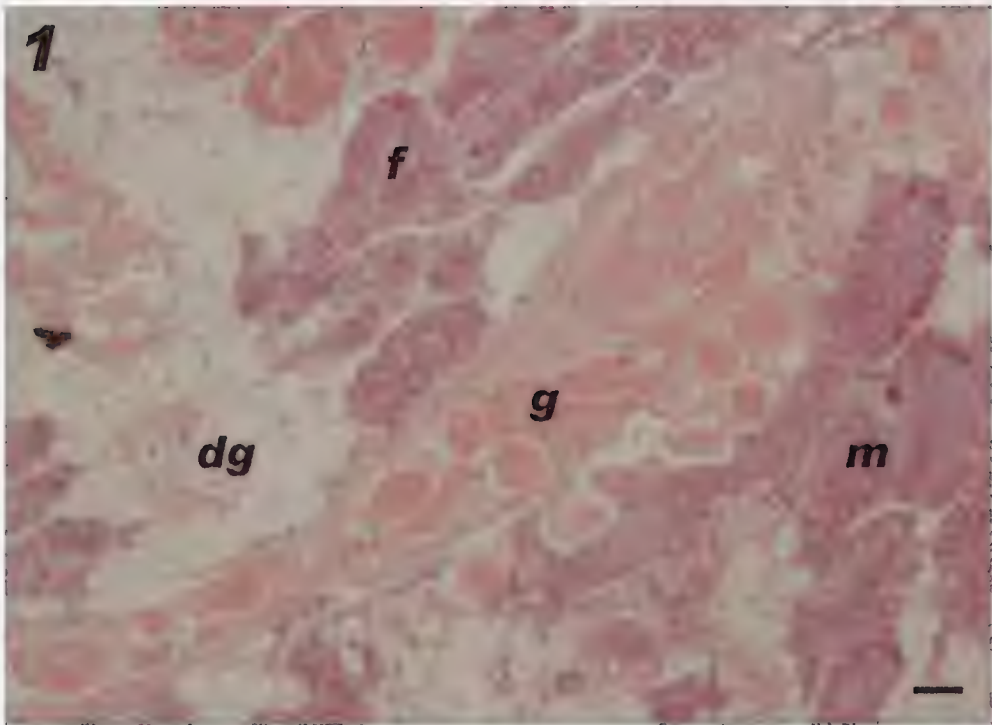
The sex of each animal could not be determined with certainty through macroscopic examination of the visceral mass. The gonadal

tissue was dispersed throughout the visceral mass surrounding the glandular digestive tissue and the gut. Male and female tissues were hardly distinguishable. Female tissue formed orange clusters predominating over the white male tissue. After microscopic analyses, all specimens of *A. cygnea*, with only one exception, were confirmed to be hermaphroditic, with both female and male gametes being observed (Fig. 1). The separately organized female and male tissues consisted of highly branched follicles surrounded by connective tissue and haemocoel spaces. Several female acini were found connected to a single germinal duct, which was lined by a ciliated epithelium, and numerous large oocytes were found in the lumen during the summer months.

The ovarian follicles were disposed symmetrically around the distal genital duct. Oogonia were present in the follicle of *A. cygnea* throughout the reproductive cycle. They had a highly basophilic nucleus and, as a consequence, could easily be identified because of their dark purple colour. The oogenesis of *A. cygnea* was clearly divided into four stages: oogonia, oocytes, pedunculated oocytes and mature oocytes (Fig. 2), all of which were present during the summer months, when oogenesis was more intense. No specimens were found in the resting period, with gametogenesis occurring all year round, although in various stages of activity. The specimens seemed to be within three main different reproductive categories (developing, ripe, and spent), although it was not possible to distinguish exactly one from another as the development of the germ cells was not simultaneous and each follicle within the gonad was in a different stage.

Based on the examination of the gonads from the specimens collected and sacrificed at monthly intervals, from October 2004 to September 2005, we have succeeded in describing the oogenic cycle of *Anodonta cygnea* L. living in Mira Lagoon.

FIGS. 1–8. Histological sections from the gonads of a hermaphroditic *Anodonta cygnea*. Scale bar = 100 µm. FIG. 1: General aspect of the gonad showing female (f) and male (m) follicles surrounding the digestive gland (dg) and a gonoduct (g) filled with mature oocytes; FIG. 2: Follicle with undifferentiated epithelium and absent lumen enclosed by connective tissue (ct); FIG. 3: Female follicle exhibiting a regular shape, a continuous wall with thick and continuous germinal epithelium (ge) and an empty medium lumen (l); FIG. 4: Irregular-shape acinus in the centre with medium lumen. Oogonia (oo), oocytes (ov), a pedunculated (p) and a mature oocyte (mo) can be identified; FIG. 5: Acinus showing a discontinuous wall (aw) and thin discontinuous epithelium with several pedunculated oocytes (po). Within the large lumen some mature oocytes (mo) are visible. Some oocytes (ov) may be seen in the connective tissue; FIG. 6: Acinus with a small lumen. Oogonia (oo) and oocytes (ov) can be identified; FIG. 7: Acini filled with atretic oocytes (ao); FIG. 8: Haemocytes (h) in the connective tissue.



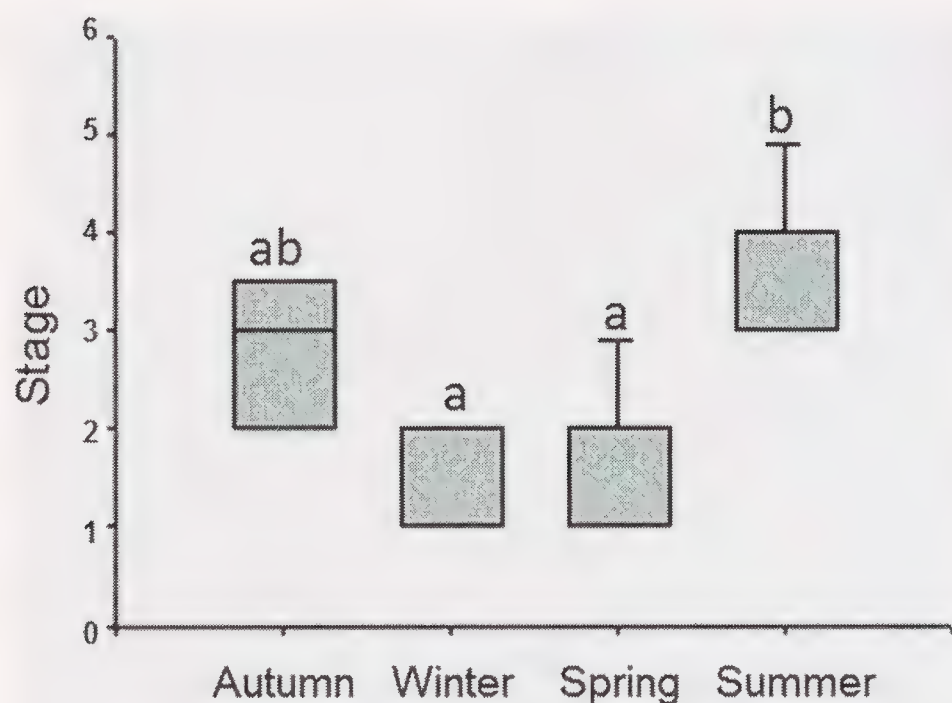


FIG. 9. Cumulative stage variation throughout the year's seasons. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. Whenever the mode is different from the median, its value is indicated within the box. In all cases analysed, the mode value was equal to the median. Distinct small letters indicate significant differences between seasons (ANOVA, $P < 0.05$).

October: Specimens showed clear signs of follicle regression (reduced gametogenesis and follicle dimensions). Some mature oocytes remained inside the lumen and the gonoduct. An invasion of haemocytes (Fig. 3) was observed outside the follicles in regression, probably having a phagocytic function against the remaining germ cells. The mothers' gills were full of developing embryos and some immature glochidia.

November: There was evidence of sexual revival since slight gametogenic activity was present. The germinal epithelium started to grow towards the centre of the lumen, which contained some oocytes with clear signs of cytolysis (Fig. 4). The first mature glochidia appeared inside the marsupial gills.

January and February: Episodes of atresia were observed more often than in the previous month. Growth of the germinal epithelium was also more evident (Fig. 5). Mature glochidia were very abundant inside the marsupial gills.

March: Acini were smaller and many had no lumen (Fig. 6) since they were all filled with germinal epithelium that often surrounded the many atretic oocytes and mature oocytes. The mothers' gills contained glochidia only in the central area; the external sections were almost empty.

April: Oogonia and previtellogenic oocytes appeared in the follicular wall of female acini. No glochidia could be found inside the mothers' gills.

May: Germinal follicles were in an active stage of development. Oogonia developed into immature oocytes, and these started to enlarge.

June: Follicle size increased and occupied the entire tissue. Germinal cells, as well as free oocytes, were present in all phases of gametogenesis (Fig. 7).

July: Follicles were almost full of ripe gametes. A decrease was also observed in the proportion of oogonia, previtellogenic and vitellogenic oocytes attached to the wall.

August: The germinal epithelium often consisted of a single discontinuous layer (Fig. 8). Many mature oocytes filled the lumen. The gonoducts appeared to be full of gametes.

September: The germinal follicles were still evident but almost empty. Once ripeness was reached, empty spaces were observed in the follicular lumen, and a lower density of free ripe oocytes was observed. Gamete production ceased and signs of gamete reabsorption were evident. It was difficult, however, to distinguish between this stage and the previous one, since gametogenesis took place very quickly, and the gametes were released in increasing quantities as they matured.

Quantitative Analysis

The overall quantitative data, analysed through the cumulative index, revealed the existence of significant variations in the gonad stage along the year (Fig. 9). Stage 1 was found mainly in winter, rising in spring to stage 2 and reaching the maximum maturity stage during the summer. In autumn, a non-significant decrease was observed. Statistically significant differences were observed not only between winter and summer, as expected, but also between spring and summer (Fig. 9.).

Seasonal stage variations were also analysed for each individually quantified cellular feature, and significant differences were found in five of them, namely: follicle wall, epithelium continuity, epithelium thickness, cell types of the epithelium, and lumen size (Fig. 10.). In these particular cases, except for the cell type within the epithelium, the variation pattern along the year was similar to that observed in the cumulative index, that is, lower develop-

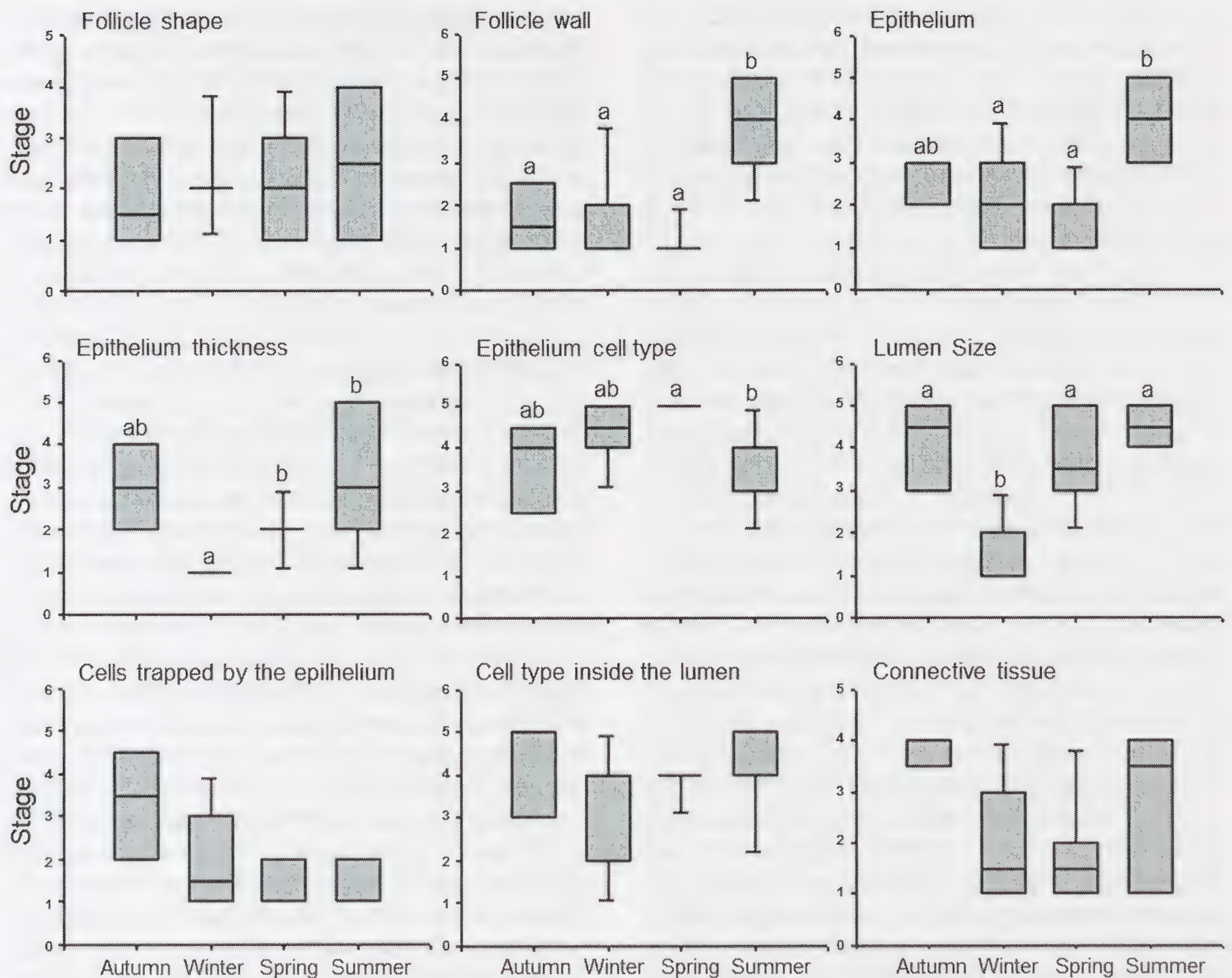


FIG. 10. Seasonal variation of each analyzed parameter relative to female follicles. The boundary of the box closest to zero indicates the 25th percentile, a line within the box marks the median, and the boundary of the box farthest from zero indicates the 75th percentile. Whiskers above and below the box indicate the 90th and 10th percentiles. In all cases analysed, the mode value was equal to the median. Distinct small letters indicate significant differences between seasons (ANOVA, $P < 0.05$).

ment stages were found in winter and these differed significantly from the higher levels of maturity reached in summer. On the other hand, maturity of the cell types within the epithelium was significantly higher in spring, and dropped in summer.

Along the year, the regularity of the follicle shape, the cell types found inside the acini lumen and the characteristics of the connective tissue also showed a variation pattern similar to that of the cumulative index, although no statistically significant differences were noted. Unlike the other characteristics described above, the cells trapped within the acini epithelium presented higher stages of maturity in autumn.

DISCUSSION

Our histological analysis has determined that the majority of *A. cygnea* are hermaphrodites. To date, eight species of freshwater mussel have been described as commonly hermaphroditic and 41 species as occasionally hermaphroditic (Henley, 2002). However, occurrence of hermaphroditism can vary among conspecific populations. Heard (1975) and Kat (1983) examined specimens of *Utterbackia imbecillis* and concluded that they were gonochoristic, whereas Sterki (1898a, b) and van der Schalie (1966) stated that *U. imbecillis* was normally hermaphroditic. According to the system described by Coe (1943), the normally

hermaphroditic classification recognizes that mussels may be functional hermaphrodites (Henley, 2002). Bloomer (1930, 1934, 1940) also investigated the sex of *A. cygnea* in the British Isles and stated that hermaphroditism was quite common in *A. cygnea* and showed different phases, the extent of which has only partly been explored.

Hermaphroditism among bivalves may occur in two forms, determined by functionally different mating systems. Sequential hermaphrodites rely on a mate (if they cannot store sperm), whereas simultaneous hermaphrodites may fertilize their own eggs. Unlike the gonochoristic population described by Galhano & Silva (1983), all *A. cygnea* examined were simultaneous hermaphrodites with prevailing female tissue. Both types of gamete develop in the same gonad. In accordance with previous reports (Heard, 1975), most specimens of *A. cygnea* showed intermingled male and female acini, dispersed throughout the viscera and without clear gonadal organization. In addition, we found some oocytes surrounded by spermatozoa inside a female follicle, suggesting the possibility of intrafollicular fertilization – a phenomenon described for other freshwater bivalves that may become self-fertilizing hermaphrodites when the population density is low (van der Schalie, 1970; Kat, 1983; Bauer, 1987; Downing et al., 1989, 1993; Araújo & Ramos, 1997). In addition to populations with a small number of individuals, hermaphroditism may also occur in populations living in stagnant water (Kat, 1983; Kükenthal et al., 1986). These are aspects that, with respect to the mussel population from Mira Lagoon, may explain the hermaphroditic condition of these specimens. In fact, anodontine species are known for the great plasticity of their life history strategies (Heard, 1975).

The mussel *A. cygnea* is generally a long-term brooder (bradytic). Spawning in late summer or fall and brooding along winter, it releases the glochidia in the following spring (Galhano & Silva, 1983). This fact has been confirmed by our study showing that *A. cygnea* has long gametogenesis and spawning periods. Nevertheless, eggs appeared in the marsupial gills in October and were brooded until the middle of April, thus suggesting a delay in the reproductive cycle.

The factors controlling the timing of gametogenesis and spawning periods in *A. cygnea* are not well known, but temperature, photoperiod and food supply are considered the most important environmental factors in the regulation

of gamete maturation and spawning in bivalves (MacDonald & Thompson, 1985; Pearse et al., 1991; Fabioux et al., 2005). *Anodonta cygnea* seems to spawn in late summer and the beginning of autumn, which correlates with the highest concentration of phytoplankton and warmest temperature found during the year (Lima et al., 2004). Spawning of *A. cygnea* does not occur in one coordinated event; instead, it takes place intermittently over a period of approximately two months (Lima, unpublished). Indeed, according to several authors, the most common spawning pattern is its sporadic occurrence over a number of weeks (Waltz, 1978; Sprung, 1983). This type of pattern is probably advantageous to the mussel population because intermittent spawning would greatly reduce the chance of catastrophic mortality due to some environmental disturbance, which could more easily occur if there was only a single larval class. Further substantiation of sporadic spawning is shown by the fact that the development of gametes within a given *Anodonta* specimen is asynchronous, that is, not all gametes within a mussel reach maturity in unison, suggesting that gamete release may occur several times over the spawning season, which generally spans the summer months.

This paper briefly describes the histological characteristics of developing oocytes that were assigned to four stages. In *A. cygnea*, as in other unionids, oogonia turned into early vitellogenic oocytes, which subsequently grew within follicles, formed vitellogenic oocytes, entered late vitellogenesis, underwent maturation and finally ovulated. During all these phases, the changes were similar to those previously reported for other unionids (Grande et al., 2001; Henley, 2002; Çek & Sereflisan, 2006; Chatchavalvanich et al., 2006). Mature oocytes are discharged from the female gonad via the germinal duct and genital aperture into the suprabranchial chamber and eventually the gill. The mature ovum is then fertilized by sperm, which enter the gill via the incurrent siphon. The ciliated epithelia lining the female germinal duct found in *A. cygnea* helps propel the gametes along its length, as described for other mussels (Chatchavalvanich et al., 2006).

Egg maturation involves the simultaneous enlargement of the ovum and the expansion of the vitelline membrane to form the vitelline space. The highest proportion of mature eggs was observed in summer samples (July–August). Gonad development seems to occur practically all year long with no resting period.

As shown by the results, gametogenesis ceases at autumn, but is reinitiated almost immediately. Early gamete development coincides so closely with the completion of the previous spawning season that acini containing both relict gametes from the previous year and newly synthesized ones for the new season are common. Year-round spermatogenesis and oogenesis have also been observed in *Anodonta imbecilis* Say (Heard, 1975) and six species of *Elliptio* (Heard, 1979).

The presence of oocytary atresia, observed during winter, may have been caused by: (1) a limited capacity of the follicle and a control mechanism governing the number of cells; (2) a self-cleaning process at the end of a gametogenic cycle, in preparation for the next cycle; and (3) a response to environmental or contaminating stress conditions (Motavkine & Varaksine, 1989). Lysis starts with the action of lysosomes in the ripe oocytes (Pipe & Moore, 1985) and its resulting products may be resorbed *in situ* via haemocytes, the auxiliary cells or the epithelial cells in the gonoduct (Lubet et al., 1987a, b; Pipe, 1987; Dorange, 1989). Oocytary lysis may have its counterpart in males, although the phenomenon may not be perceived due to the small size of spermatozoa. Indeed, Bayne et al. (1978) observed masses of haemocytes inside developing follicles in male acini of *Mytilus edulis* Linnaeus, which may indicate spermatozoa degeneration as well. The role of haemocytes as macrophages, during the resorption of products from the lysis of oocytes and spermatozoa, was reported for the first time in *Pecten maximus* Linnaeus (Dorange, 1989; Dorange & Le Pennec, 1989).

Degeneration and re-initiation occurred at the same time in the gonad where two generations of germ cells could be simultaneously observed. We could also confirm this occurrence inside *A. cygnea* female acini. These observations seemed to indicate that gamete resorption is a dispensable step for the onset of a new reproductive cycle.

The subjectivity that may arise in qualitative analysis classification can be avoided by applying a quantitative analysis which removes much of the subjective error from data collection and also provides better opportunities for descriptive and comparative analysis, as shown by Wilson & Simons (1985), Choi & Chang (2003) and Callil & Mansur (2007).

Previous studies have been conducted on aspects of the reproductive cycle of the swan mussel in different parts of the world (Giusti et

al., 1975; Galhano & Silva, 1983), but these research works make no correlation between the quantity of follicular components or acinus and gametogenic cycle stages. The histological classification system was reasonably effective in describing the general reproductive trends, but it did not provide the same level of precision or capacity in comparison with the quantitative methods. Thus, our study is the first to provide a specific scoring system for gonadal development of this bivalve and, although the resulting classification scale is specific to *Anodonta cygnea*, the method can be extended to other species.

Of the quantified parameters, the continuity of the follicle wall, the continuity and thickness of the germinal epithelium and the lumen size followed the same variation pattern of the cumulative index, throughout the year. On the other hand, the epithelial cell types varied in a different way, although with significant differences among themselves. Buchanan (2001) examined gonad histological sections in order to study the annual reproductive cycle in both sexes of *Perna canaliculus* Gmelin both qualitatively, using a classification and scoring system, and quantitatively, using image analysis technology. He found that the follicle cover (FC) and gamete cover (GC) measurements of gonad morphological status provided a good description of the annual reproductive cycle. In fact, power analysis demonstrated that GC was the most robust means to analyse reproductive condition. On the other hand, the proportion of the follicle filled with gametes (G/F) was not a good indicator of the annual reproductive cycle, since this parameter did not vary greatly during spawning and redevelopment. Only at the extremes of spawning and maturity did G/F fluctuate significantly. Comparatively, it would be plausible to consider the hypothesis that subsequent works should apply the quantification of only those features found to be most outstanding in this study, because they vary in the same way as the cumulative index. In this case, however, the precision of the quantitative methods would be lost since the other features make the difference between close levels.

As gametogenesis in the swan mussel occurs all year round, we may say that it seems possible to artificially induce gonad maturity and subsequent spawning and fertilization. Therefore, the next step towards successful artificial culture is to study a method which allows us to obtain several larval classes throughout the year.

ACKNOWLEDGMENTS

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LIGHT AND TRANSMISSION ELECTRON MICROSCOPY OF TWO
SPERMATOGENIC PATHWAYS AND UNIMORPHIC SPERMATOOZOA IN
VENUSTACONCHA ELLIPSIFORMIS (CONRAD, 1836) (BIVALVIA: UNIONOIDA)

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ABSTRACT

This study followed the typical and atypical pathways of spermatogenesis in *Venustaconcha ellipsiformis* (Conrad, 1836) (Bivalvia: Unionoida: Unionidae), during an entire year and documents the developmental stages of both using light and transmission electron microscopy. The spermatozoa produced by both pathways were essentially morphologically identical and contained a single acrosomal vesicle. Production of spermatozoa in the two pathways overlapped from May to October, and vitellogenic eggs were present over this same time period. Atypical spermatogenesis also occurred from November through April. The typical pathway involved the mitotic and meiotic divisions that include spermatogonial cells, primary and secondary spermatocytes, spermatids and mature spermatozoa. In contrast, atypical spermatozoa appeared to be produced in a pathway that included spermatozoa morulae and spermatids produced from spermatogonial cells located in Sertoli cells. Large amorphous inclusions (AI) observed in Sertoli cells, and apparently acting as secondary lysosomes in digesting morulae, are described for the first time. The morphology of the AI was variable, with the AI sometimes containing structures that were organelle-like in appearance and some of the AI appeared to merge together. Phagocytes associated with both the typical and atypical pathways, but distinct from Sertoli cells, were observed clearing the acini of cellular debris. Immunoelectron microscopy detected the presence of both female-transmitted (F) and male-transmitted (M) mitochondria in the spermatozoa present in July (when fertilization takes place). We propose that the necessity of producing spermatozoa with F and M mt genome-bearing mitochondria, to maintain dioecy and 50 : 50 sex ratios in freshwater bivalve populations, accounts for the > 200 million year maintenance of two spermatogenic pathways in the Unionoida.

Key words: atypical and typical spermatogenesis, doubly uniparental inheritance of mitochondrial DNA (DUI), ultrastructure, Unionoida, *Venustaconcha ellipsiformis*.

INTRODUCTION

The Mollusca is one of several higher taxa in which heteromorphic spermatozoa are found (Buckland-Nicks & Chia, 1986; Jespersen et al., 2001; Buckland-Nicks & Thompkins, 2005). Outside the Mollusca, other taxa that are known to produce heteromorphic male gametes are plants, arthropods, annelids, nematodes, echinoderms and vertebrates (Cook & Wedell, 1999; Ferraguti et al., 2002; Till-Bottraud et al., 2005; Paccagnini et al., 2006; Immler, 2008). Many gastropod species produce spermatozoa by two spermatogenic pathways referred to as typical and atypical (Dohmen, 1983). In the typical pathway, fertilizing spermatozoa develop

by a series of mitotic and meiotic cell divisions involving spermatogonial cells, primary and secondary spermatocytes, spermatids and mature spermatozoa. Other spermatogenic pathways that result in non-fertilizing spermatozoa or in which spermatozoa are produced by stages that differ from the typical pathway stages, are called atypical. Spermatozoa produced by an atypical pathway are often morphologically distinct from typical spermatozoa (Heard, 1975). When present together, the two morphologically distinct spermatozoa are usually referred to as eusperm or fertilizing spermatozoa (typical) and parasperm, or non-fertilizing spermatozoa (atypical) (Healy & Jamieson, 1981). Functions for the atypical spermatozoa

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have been reported as aiding in protection and transport of the typical spermatozoa to the egg, often by forming a spermatzeugmata (Nishiwaki & Tochimoto, 1969; Lynn, 1994; Cook & Wedell, 1999; Ishibashi et al., 2000; Jespersen et al., 2001; Ferraguti et al., 2002), providing nutrition to the egg, creating a hostile environment for competing spermatozoa (Gould, 1917; Kura & Nakashima, 2000; Buckland-Nicks & Thompkins, 2005; Immler, 2008) or interfering with female ability to be reinseminated with rival spermatozoa (Cook & Wedell, 1999; reviewed in Till-Bottraud et al., 2005). Spermatzeugmata have also been reported as containing atypical spermatozoa (Nishiwaki & Tochimoto, 1969; Waller & Lasee, 1997), although some spermatzeugmata contain fertilizing spermatozoa (Ó Foighil, 1989). Various pathways of spermatogenesis have been described for species that produce heteromorphic spermatozoa including separate spermatogonial cells (Buckland-Nicks & Thompkins, 2005), one type of spermatogonial cell (Ferraguti et al., 2002; Paccagnini et al., 2006) and accessory cells (Reinke, 1902), while in some cases, the pathway is not stated (Cook & Wedell, 1999; Jespersen et al., 2001).

As is noted in Buckland-Nicks & Chia (1986), several terms have been used to describe the somatic cells that are located on the wall of a testicular acinus and which extend into its lumen. In the present study, in agreement with Syasina (1992), whose review is on molluscs, these cells will be referred to as "Sertoli cells". Sertoli cells have been associated with spermatogenesis in many different animals (Roosen-Runge, 1977; Reunov et al., 2004; Smita et al., 2005; Sasso-Cerri et al., 2006; Wang et al., 2006; reviewed in Syasina, 1992), including molluscs. Their roles in molluscs have been noted as that of nursery cells, which provide nutrients to developing spermatozoa, phagocytosis and destruction of residual bodies and abnormal or unspawned spermatozoa and contributions to hormonal control of spermatogenesis and maintenance of the blood-testis barrier (Buckland-Nicks & Chia, 1986; Pipe, 1987; Sousa et al., 1989; Dorange & LePennec, 1989; Rocha & Azevedo, 1990; Eckelbarger & Davis, 1996; Erkan & Sousa, 2002; Syasina, 1992; Kiyomoto et al., 1996). Sertoli cells have also been documented as containing spermatozoa morulae (Matos et al., 1998). Spermatozoa morulae, which are reported to initially contain spermatids (Matos et al., 1998), or possibly various cells of the typical spermatogenic process (Chatchavalvanich et al., 2006), are

widespread in the Unionoida (Coe & Turner, 1938; Bloomer, 1946; Heard, 1975; Jones et al., 1986; Kotrla, 1988, 1989; Haggerty et al., 1995; Byrne, 1998; Garner et al., 1999; Shiver, 2002; Cek & Sereflisan, 2006; Chatchavalvanich et al., 2006). Spermatozoa morulae in unionoid bivalves have been observed in species representing all unionoid families and they are always associated with the atypical pathway of spermatogenesis (Heard, 1975, unpublished observations; Kotrla, 1988, 1989).

Bivalve species in the order Unionoida typically possess separate sexes (dioecy) and two highly unusual reproductive characteristics: high fidelity doubly uniparental inheritance of mitochondrial (mt) DNA (DUI; Breton et al., 2007) and two distinct spermatogenic pathways (Heard, 1975; Kotrla, 1988, 1989). DUI is a unique form of mtDNA transmission that has been detected only in mytiloid, veneroid and unionoid bivalves (Breton et al., 2007). Under DUI, females pass on their F-type mitochondrial DNA (mtDNA) to both sons and daughters. This is similar to transmission occurring under strict maternal inheritance of mtDNA (SMI) in all other animal species. However, in contrast to the situation present in other animals, male mussels transmit their M-type mtDNA, via the five mitochondria comprising the spermatozoon mid-piece, only to sons. Male somatic tissue typically contains predominantly the mother's F-type mtDNA, whereas male gametes exclusively contain the M-type mtDNA inherited from the father. The strict preservation of this mode of inheritance in dioecious unionoid species has produced two parallel but highly divergent mtDNA lineages that originated > 200 million years ago (Breton et al., 2007, 2011; Doucet-Beaupré et al., 2010). The DUI system in bivalves not only challenges our traditional view of strict maternal inheritance of mtDNA, it also demonstrates remarkable genetic novelties in animal mt genomes: an extension (MCOX2e) of the cytochrome c oxidase subunit II protein (MCOX2) that is only encoded by the unionoid bivalve M mt genomes and two additional, gender-specific and rapidly evolving mt-encoded proteins (i.e., protein genes other than the typical set of 13 usually found in animal mtDNAs), whose functions remain unknown (the *FORF* and *MORF* proteins in the unionoid F and M mt genomes, respectively). An antibody to the extension region of the MCOX2 protein and an antibody to the *FORF* protein have been produced (Chakrabarti, 2006, 2007; Breton et al., 2009, 2011).

In addition to the presence of DUI in dioecious unionoid species, morulae, which are associated with the atypical pathway of spermatogenesis, are present in the testicular tissues of all species that have been thoroughly studied, as cited above. The genesis and function of these structures, broadly distributed within the Unionoida, are unclear but previous authors have stated that these structures develop into mature spermatozoa (Kotrla, 1988; Matos et al., 1998). Therefore, the phenomena of DUI and two distinct spermatogenic pathways, typical and atypical, are inextricably linked to unionoid spermatogenesis.

Phagocytic and lysosomal activities associated with spermatogenic tissues are common. As mentioned above, Sertoli cells have been observed engaged in these processes in various animal taxa (Reunov et al., 2004; Smita et al., 2005; Sasso-Cerri et al., 2006; Wang et al., 2006), including molluscs (Buckland-Nicks & Chia, 1986; Pipe, 1987; Dorange & LePennec, 1989; Sousa et al., 1989; Rocha & Alzevedo, 1990; Syasina, 1992; Eckelbarger & Davis, 1996; Kiyomoto et al., 1996; Erkan & Sousa, 2002). Phagocytes that are distinct from Sertoli cells are well documented in a variety of organisms including leeches (Bonet & Molinas, 1991), caecilians (Smita et al., 2005), lugworms (Pacey & Bentley, 1992), sea urchins (Reunov et al., 2004), and molluscs (López et al., 1997).

Spermatozoon ultrastructure has been considered a useful character for evolutionary comparisons with acrosomal ultrastructure being of particular interest. For example, studies between spermatozoa of *Velesunio ambiguus* (Philippi, 1847) (Unionoida: Hyriidae) and trigonoid spermatozoa (*Neotrigonia bednalli* [Verco, 1907]) established similarities of multiple vesicles within the acrosome of these two animals leading to the proposal that the Trigonoida and Unionoida could have a relatively close phylogenetic relationship (Healy, 1989). This phylogenetic hypothesis has been corroborated by subsequent studies (Hoeh et al., 1998, 2009; Graf & Cummings, 2006). Some freshwater clams produce spermatozoa with a single acrosomal vesicle and this characteristic is generally designated a plesiomorphic character state (Franzen, 1956; Trimble & Gaudin, 1975). However, acrosome development has been demonstrated to take many forms in bivalves (Reunov et al., 1999) and the acrosome structure itself could be related to aspects of egg-spermatozoa interaction during fertilization as well evolutionary history (Popham, 1979).

The present report describes, in detail, the typical and atypical pathways of spermatogenesis in *Venustaconcha ellipsiformis* (Conrad, 1836) and their temporal overlap with each other and with the presence of vitellogenic eggs. It is based on consecutive, monthly samples from an entire year using light and transmission electron microscopic observations. Additionally, immunoelectron microscopy (IEM) techniques demonstrated the presence of both the M and F mt proteins in unionoid spermatozoa. The results from the IEM experiments, together with the morphological evidence from the typical and atypical spermatogenic pathways, suggest a hypothesis for the > 200 million year persistence of dual spermatogenic pathways in unionoid bivalves.

MATERIALS AND METHODS

Specimens

Because it was known from preliminary studies that *Venustaconcha ellipsiformis* (Conrad, 1836) exhibited both typical and atypical spermatogenesis pathways and that frequent sampling was required by the aims of this study, this species was selected due to its proximity to Saginaw Valley State University. Monthly collections of *V. ellipsiformis* were made from the Sugar River in Gladwin County, Michigan, between January and December 2006, with the exception of March (Fig. 1). Animals were placed in a container of ice and immediately transported to the laboratory where male and female gonadal tissue was excised and processed as described below. All protocols complied with appropriate animal care committee policies at Saginaw Valley State University and adhered to the legal requirements established in the United States regarding animal use and care in research.

Conventional Transmission Electron Microscopy

Tissues were placed in 2.5% glutaraldehyde in mussel buffer (Misamore & Lynn, 2000). They were stored at 4°C for up to several months. Post fixation was in 0.5% OsO₄ in buffer for 1 hr, also at 4°C. Both fixatives were followed by 2 x 10 min buffer washes. Dehydration was in a graded series of acetone and tissues were embedded in Spurr's resin. Sections were cut at 70 nm, stained with uranyl acetate and lead citrate and viewed with a JEOL 1400 transmission electron microscope at 80 KV.

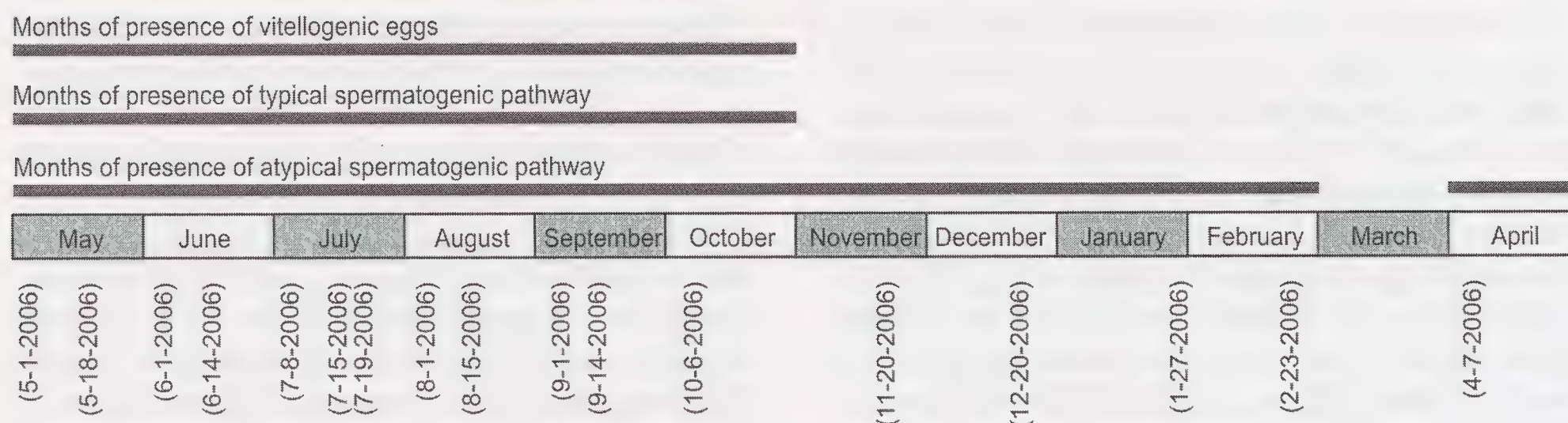


FIG. 1. Phenology of the presence of vitellogenic eggs and typical and atypical pathway sperm; note that no sample was taken in March. Sampling dates are in parentheses.

Light Microscopy

For some of the light microscopy, sections of the Spurr's embedded material were cut at 0.5 μ m and stained with toluidine blue. Others were fixed with 1.5% buffered formalin and prepared for embedding in paraffin according to standard procedure (Humason, 1967). Sections were cut at a thickness of 7 μ m and stained with the Feulgen's DNA-specific protocol and counter-stained with 0.5% fast green (Humason, 1967). Ovarian sections were stained with hematoxylin and eosin (Humason, 1967). Images were viewed under a Nikon Optiphot and collected using Image Pro 5.1.2 software.

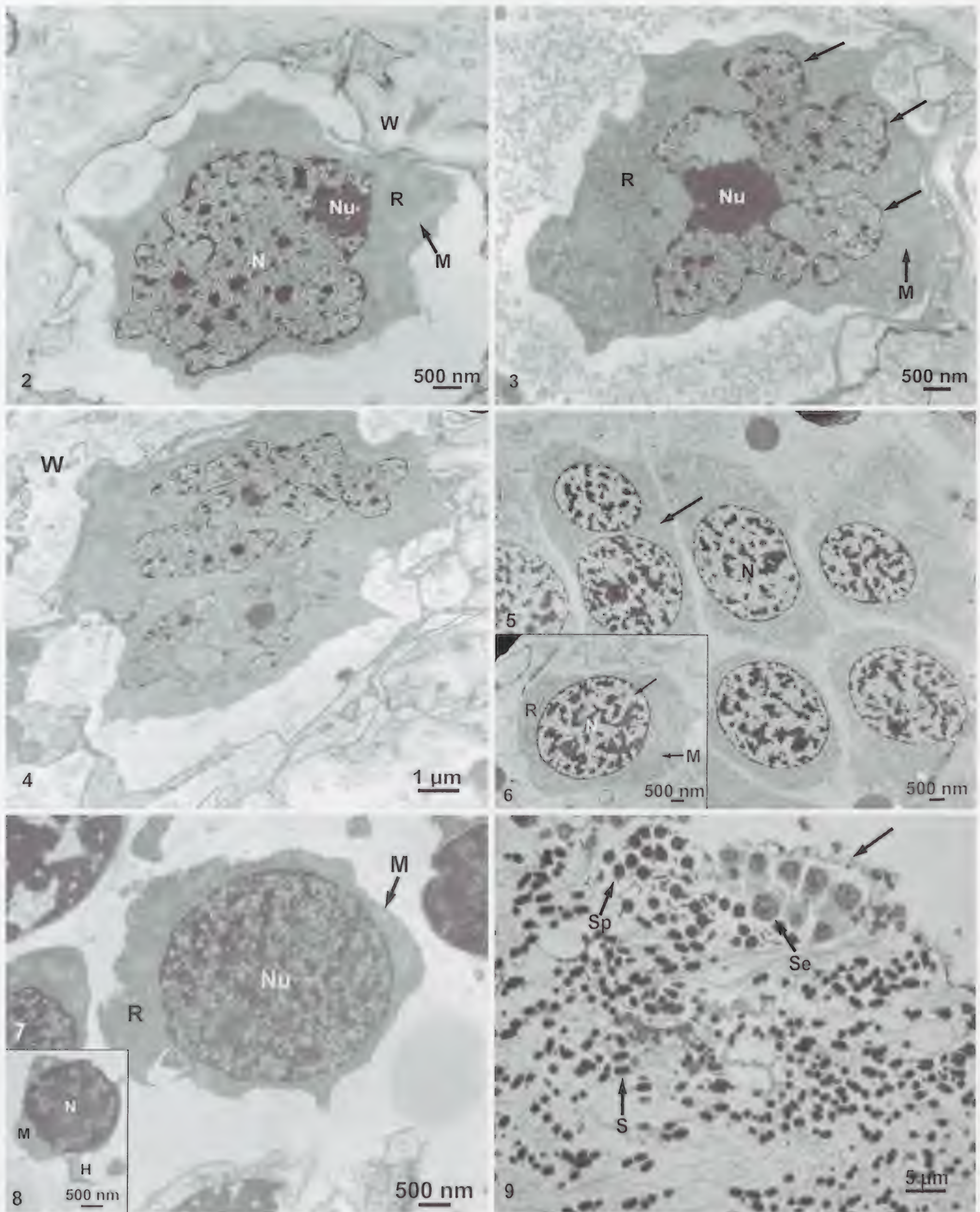
Immunoelectron Microscopy

Bivalve tissues were fixed in 1.0% glutaraldehyde and 4.0% paraformaldehyde in 0.1 M phosphate buffer, pH 7.2, for 4 hr and washed 3 x 10 min in buffer and embedded in LR White. Tissues were sectioned (70 nm), placed on nickel grids and incubated for 15 min in a low molecular weight blocking solution (0.05% glycine in PBS), followed by 30 min in a high molecular weight blocking solution (goat block solution, Electron Microscopy Sciences, Fort Washington, Pennsylvania) and a 5 min wash in incubation buffer (10 mM phosphate buffer, 150 mM NaCl, pH 7.4 with 0.2% BSA-c and 15 mM NaN_3). Sections were then incubated with either the MCOX2e primary antibody (1:200) or the FORF primary antibody (1 : 10) in incubation buffer for 1 hr. After 3 x 5 min incubation buffer washes, grids were placed in gold solution (10 nm gold in goat anti rabbit serum, diluted 1 : 20 in incubation buffer, Electron Microscopy Sciences) for 2 hr. Six x

5 min incubation buffer washes were followed by 3 x 5 min PBS washes. Sections were post fixed in 2.0% glutaraldehyde in PBS for 5 min, washed in PBS for 5 min x 5 in dH_2O for 2 min each. Sections were post stained in uranyl acetate for 15 min and lead citrate for 1 min and viewed with a JEOL 1400 or 100 CX TEM at 80 KV. Control IEM protocols omitted the primary antibodies.

Figure Abbreviations

A	acrosome
AI	amorphous inclusion
C	collar of midpiece
E	egg
ER	endoplasmic reticulum
F	flagellum
H	cytoplasmic hook
L	lipid
Lu	lumen
Ly	lysosome
M	mitochondrion
Mo	spermatozoon morula
N	nucleus
Nu	nucleolus
P	primary spermatocyte
Pc	phagocyte
R	ribosomes
S	spermatozoon
SC	Sertoli cell
SCI	spermatozoa cluster
Se	secondary spermatocyte
SG	spermatogonial cell
SGG	spermatogonial cell group
Sp	spermatid
St	stock
V	vacuole
W	acinus wall



FIGS. 2–9. Typical spermatogenic pathway. FIG. 2: Typical pathway spermatogonial cell at acinus wall; FIG. 3: Typical pathway multi-lobed (arrows) spermatogonial nucleus; FIG. 4: Typical pathway syncytium prior to break up into individual cells; FIG. 5: Typical pathway syncytium after break up into individual cells; division of cells (arrow) indicates the formation of primary spermatocytes and additional spermatogonial cells; FIG. 6: Typical pathway primary spermatocyte (arrow) with large, oblong nucleus; note less dense euchromatin (arrow) than in spermatogonial cell; FIG. 7: Typical pathway secondary spermatocyte with spherical nucleus containing highly condensed chromatin; FIG. 8: Typical pathway spermatid with mitochondria at one side of the nucleus; FIG. 9: Movement of typical pathway cells from acini wall (arrow); spermatogonial cells and primary spermatocytes are not in view.

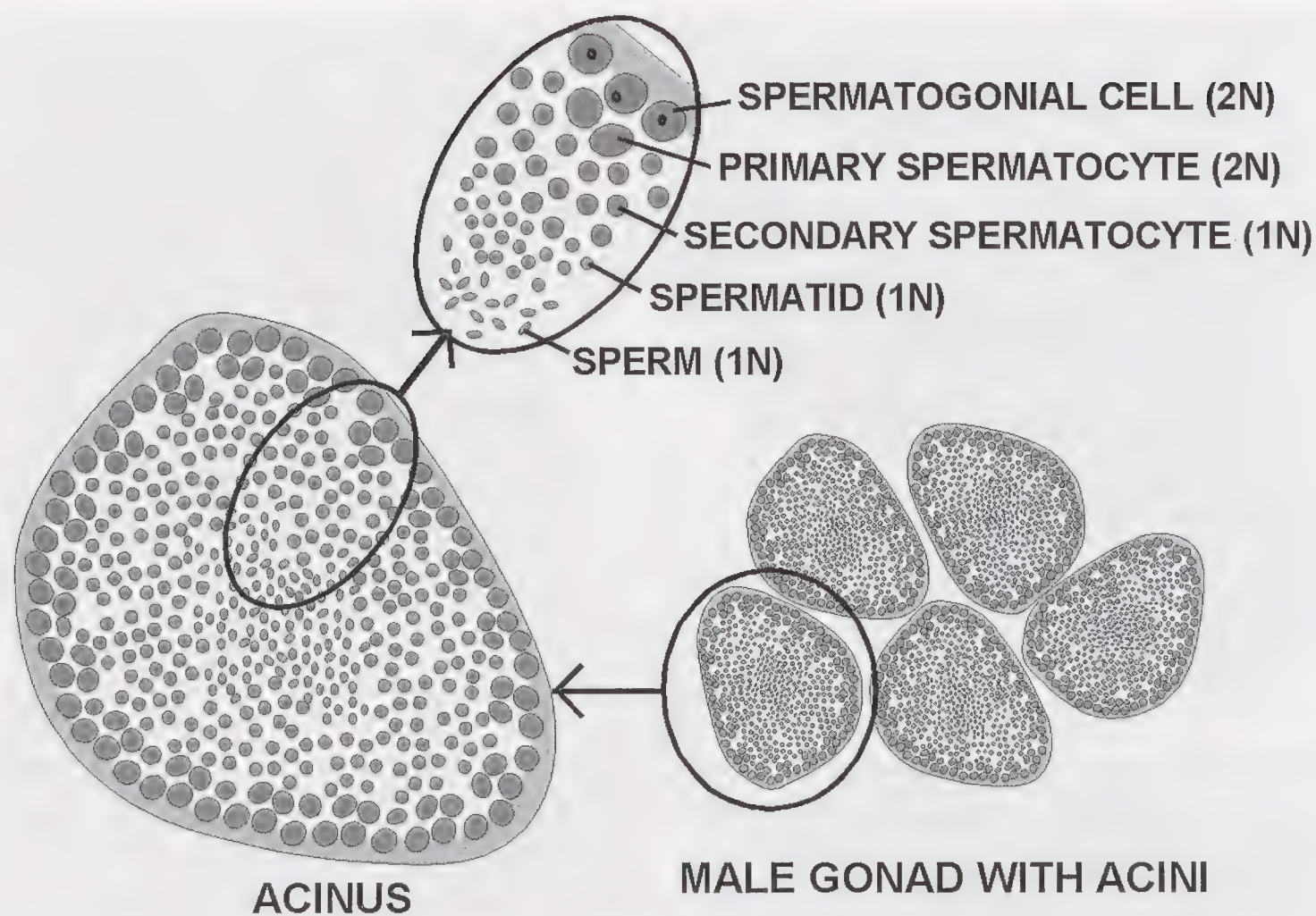


FIG. 10. Line drawing of typical pathway spermatogenesis compiled from several light and electron micrographs.

RESULTS

Typical Spermatogenic Pathway

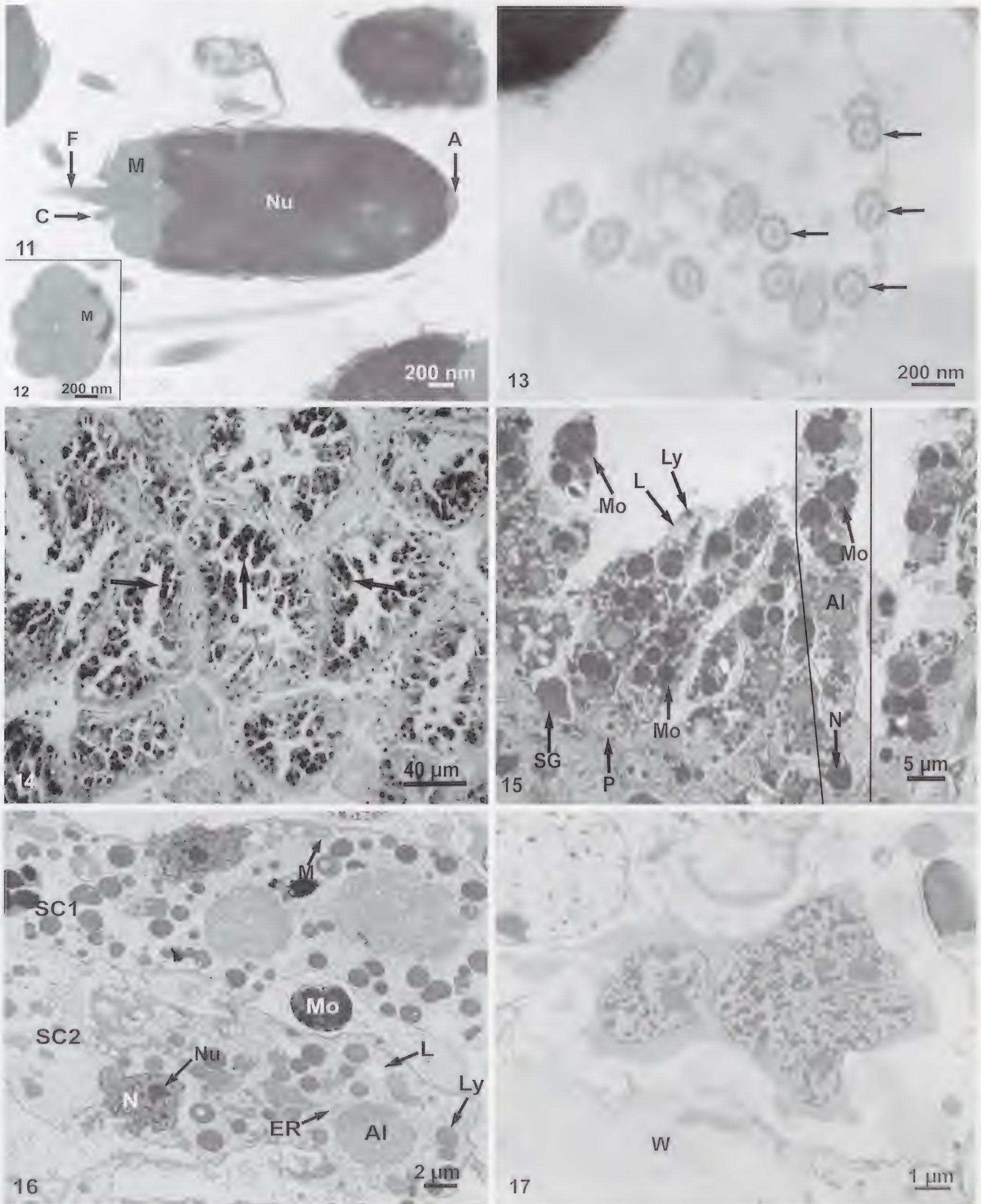
The architecture of the testes in unionoid bivalves has been described elsewhere (Heard, 1975). In *Venustaconcha ellipsiformis*, typical spermatogenesis took place in the acini. The process began in early May and ended in October (Fig. 1). Spermatogenesis originated in spermatogonial cells, which were initially located on the acinar wall (Fig. 2). Spermatogonial cells contained a large, lobed nucleus with a prominent nucleolus, semi-condensed chromatin and dense euchromatin. Also present were numerous mitochondria and ribosomes (Fig. 2). Preceding division into more spermatogonial cells and primary spermatocytes, the spermatogonial nucleus became multi-lobed (Fig. 3). Newly formed spermatogonial cells and primary spermatocytes formed a syncytium, which eventually broke apart (Figs. 4, 5). Mature primary spermatocytes contained an oblong spherical nucleus, often with no obvious nucleolus, but containing evenly distributed condensed chromatin of varying amounts. Euchromatin in primary spermatocytes was distinctly less electron dense than that of the spermatogonial cells while mitochondria and ribosomes were numerous (Fig. 6). Secondary spermatocytes had less cytoplasm than

primary spermatocytes and the nuclei were spherical with highly condensed chromatin content. Mitochondria and ribosomes remained (Fig. 7). In the spermatid stage, mitochondria were observed aggregated at one side of the nuclei and cytoplasmic hooks were common (Fig. 8). As typical spermatogenesis proceeded in May, spermatogonial cells, and all other cells produced from them, moved away from the acinar wall toward the acinar center (Fig. 9). In July, acini were filled with spermatozoa (Fig. 9). The entire process of typical spermatogenesis is summarized in Figure 10.

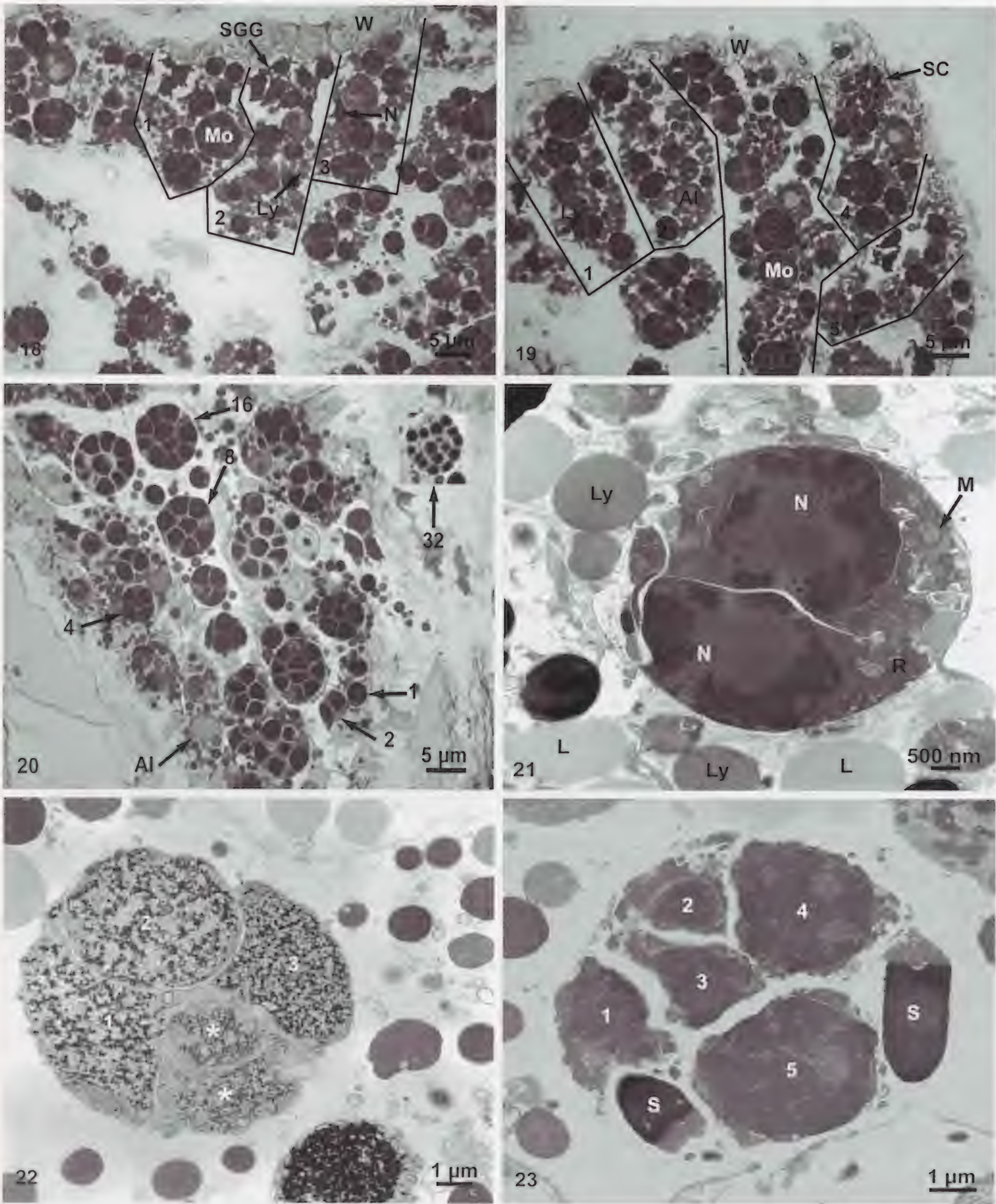
Mature spermatozoa had a bullet shaped nucleus of condensed chromatin with an acrosome containing a single vesicle (Fig. 11). Five mitochondria were observed at the posterior end of all spermatozoa studied (Fig. 12) and a single flagellum emerged through the collar of the mid-piece (Fig. 11). Axonemes contained the 9+2 arrangement of microtubules (Fig. 13). No spermatozeugmata (spermatozoa balls) were observed.

Atypical Spermatogenic Pathway and Sertoli Cells

The atypical pathway of spermatogenesis in *V. ellipsiformis* also took place in the acini but was active year round. The designation of spermatozoa observed from October – April as atypical was inferred from the lack of any



FIGS. 11–17. Typical and atypical spermatogenic pathways. FIG. 11: Typical pathway spermatozoon with bullet shaped nucleus; FIG. 12: Typical pathway spermatozoon showing five mitochondria in cross section of midpiece; FIG. 13: Cross sections of typical pathway spermatozoa axonemes showing 9+2 arrangement of microtubules (arrows); FIG. 14: Atypical pathway Sertoli cells (arrows) projected finger-like into the acini; FIG. 15: Atypical pathway Sertoli cells with one indicated inside lines; FIG. 16: Two atypical pathway Sertoli cells; FIG. 17: Atypical pathway syncytium prior to breakup into individual cells.



FIGS. 18–23. Atypical spermatogenic pathway. FIG. 18: Sertoli cells (1, 2) showing spermatogonial cell groups; FIG. 19: Later stage Sertoli cells (1, 2, 3, 4) showing high numbers of morulae and spermatozoa in clusters but absence of spermatogonial cells; FIG. 20: Atypical pathway morulae showing 1, 2, 4, 8, 16, and 32 (inset) cells; FIG. 21: Atypical pathway two-celled morula with highly condensed chromatin in the nuclei; FIG. 22: Atypical pathway four-celled morula with uncondensed chromatin in the nuclei inferring cell division within the morula; note that one of the four cells (*) had apparently begun its next division asynchronously to cells 1, 2 and 3; FIG. 23: Atypical pathway morula with five cells (1, 2, 3, 4, 5) and two mature spermatozoa (S) visible.

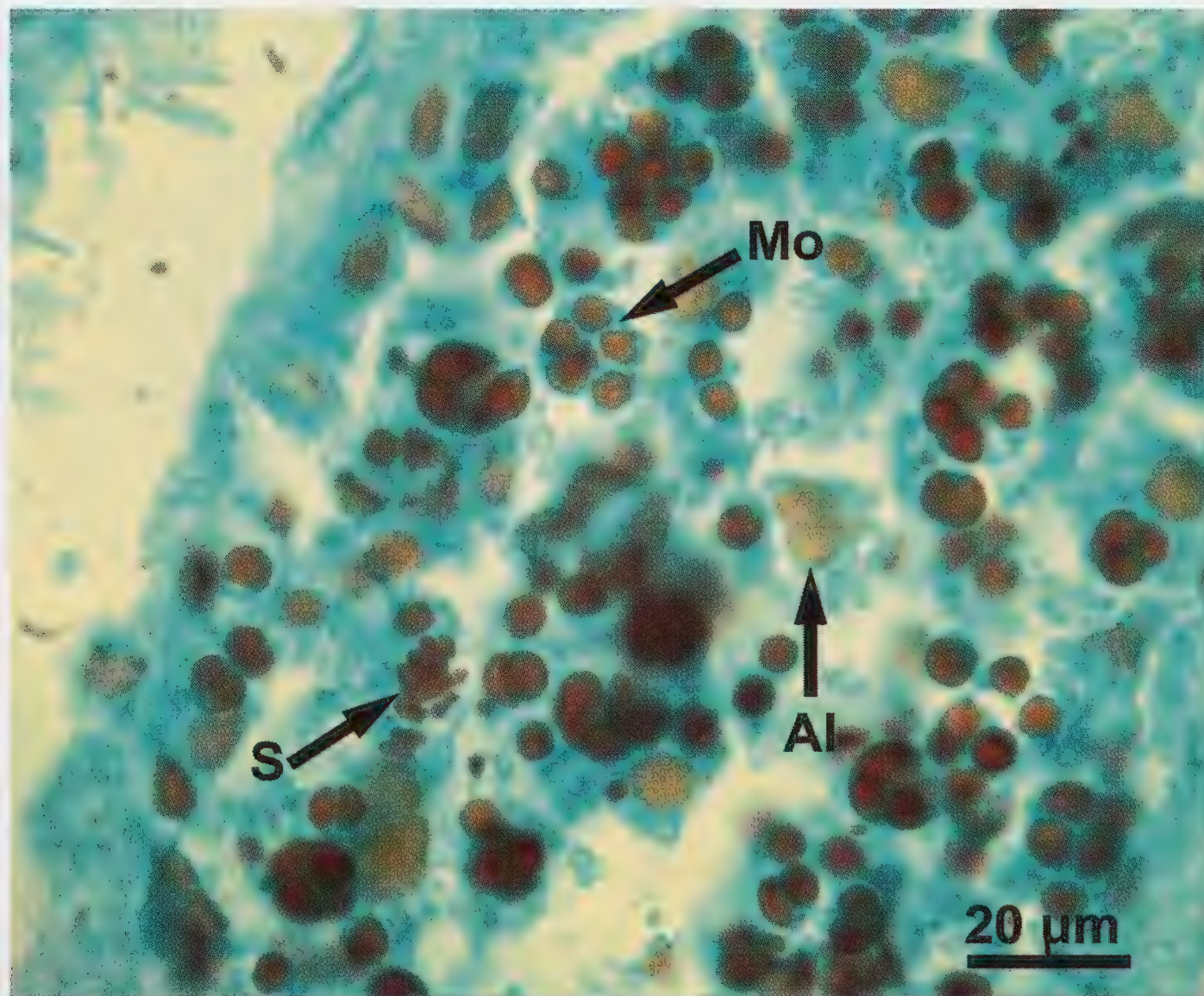


FIG. 24. Feulgen's stain on atypical pathway Sertoli cells; note positive (red) stain on morula, amorphous inclusion, and spermatozoa.

observations of activity in typical pathway spermatogonial cells beyond the occasional primary spermatocyte stage. A significant cell type involved in the atypical pathway was the Sertoli cell (Figs. 14–16). These cells were initially located at the acinar wall but developed into finger-like projections protruding into the acinar lumen as they matured (Figs. 14, 15). Syncytia, essentially identical to those of the typical pathway, were observed in Sertoli cells near the acinar wall (Fig. 17). Sertoli cells contained nuclei with an undulating outline, a nucleolus and patches of condensed chromatin dispersed throughout the nuclei (Fig. 16). Sertoli cells also displayed mitochondria, endoplasmic reticulum, lipid bodies, lysosomes, sperm morulae (see below) and amorphous inclusions (AI) (see below) (Figs. 15–17). All of these structures were identified by their morphological characteristics. No long, thin cytoplasmic extensions were present (Figs. 15, 16), nor were junctions between cells. The syncytia broke apart into groups of separate spermatogonial cells (Fig. 18) essentially identical to those of the typical pathway (Fig. 2). Some Sertoli cells contained many morulae as well as spermatozoa clusters

but no spermatogonial cells (Fig. 19, cells 1, 2, 3, 4).

Spermatozoa Morulae

Spermatozoa morulae were numerous in Sertoli cells (Figs. 15, 18, 19). Each morula contained 1, 2, 4, 8, 16 or 32 cells (Fig. 20). The numbers 8, 16 and 32 were assumed due to the thinness of the sections and the likelihood of cell division in the morulae (see below). Nuclei of the morulae cells had highly condensed chromatin with patches of lighter areas and mitochondria and ribosomes were present (Fig. 21). An intact membrane surrounded the individual cells of each morula as well as the entire morula (Figs. 21, 22). Many morulae were in close contact with lipid bodies and lysosomes (Fig. 21). Apparent stages of division of the cells in the morulae, inferred from the appearance of the uncondensed chromatin and smaller size of some of the cells, were observed (Fig. 22). All cells within a morula did not necessarily divide synchronously (Figs. 22, 23). The same Sertoli cell often contained morulae possessing different cell numbers (Figs. 15, 18, 19, 20).

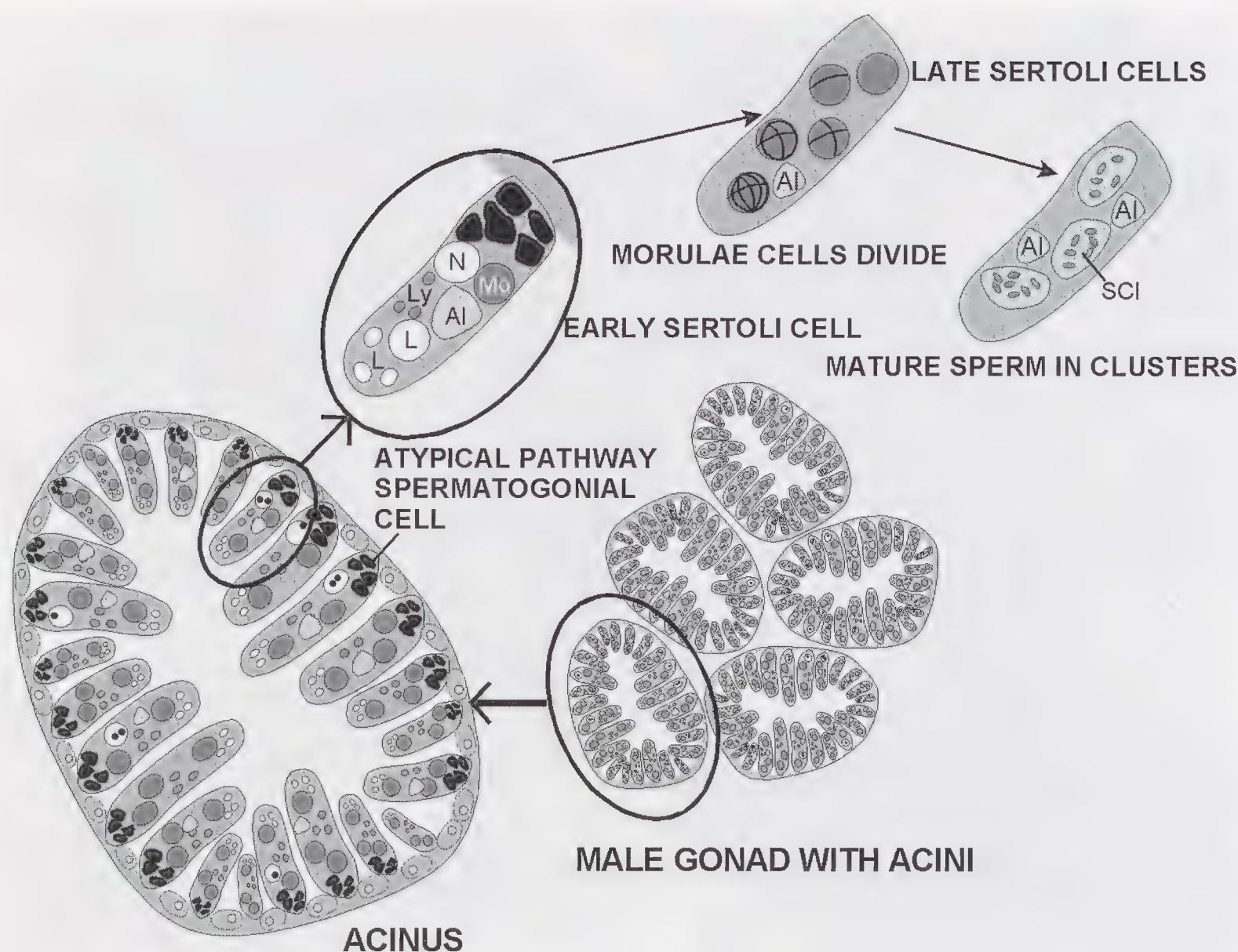


FIG. 25. Line drawing of atypical pathway spermatogenesis compiled from several light and electron micrographs.

Atypical Pathway Spermatozoa

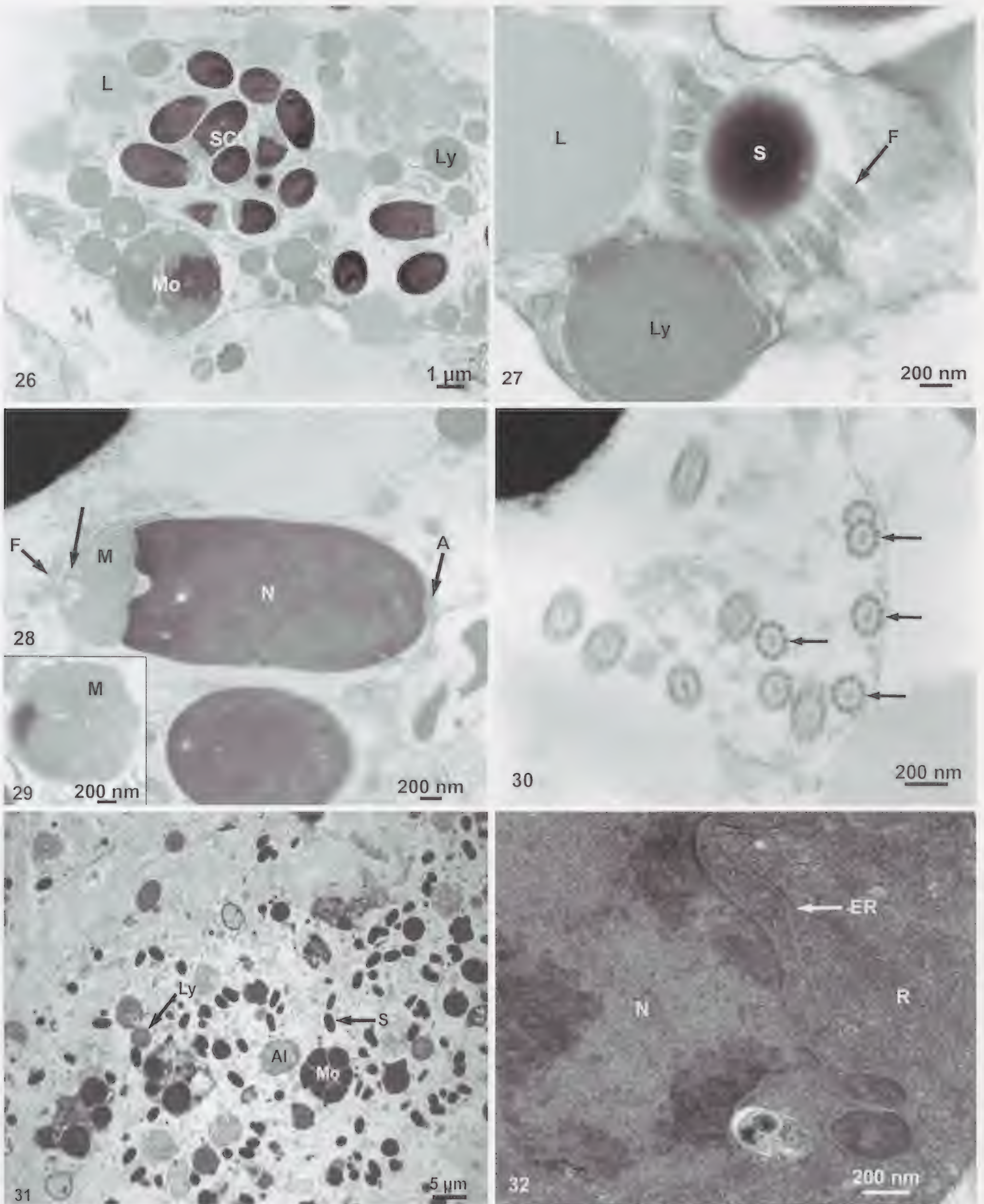
Mature atypical spermatozoa were often associated with the cells of a morula (Fig. 23) and were also located in membrane bound clusters in late stage Sertoli cells (Figs. 19, 24). The entire process of atypical spermatogenesis is summarized in Figure 25. The precise number of spermatozoa in clusters was difficult to ascertain because of the section thinness (Fig. 26). Atypical spermatozoa stained positive for DNA, as did morulae cell nuclei (Fig. 24). Flagella were observed wrapped around the spermatozoa when the spermatozoa were clustered (Fig. 27). Atypical spermatozoon structure closely matched that of the typical spermatozoa with a bullet shaped nucleus containing condensed chromatin, an acrosome with a single vesicle, five mitochondria in the mid-piece and a single flagellum which emerged through the collar of the mid-piece (Figs. 28, 29). Axonemes displayed the 9+2 configuration (Fig. 30). No spermatozeugmata (spermatozoa balls) were observed. Sertoli cells eventually broke down and released their contents into acinar lumina. At this stage, all of the various Sertoli cell organelles, morulae, Al (see below) and mature spermatozoa could be seen scattered in the acinar lumina (Fig. 31).

Amorphous Inclusions (Al)

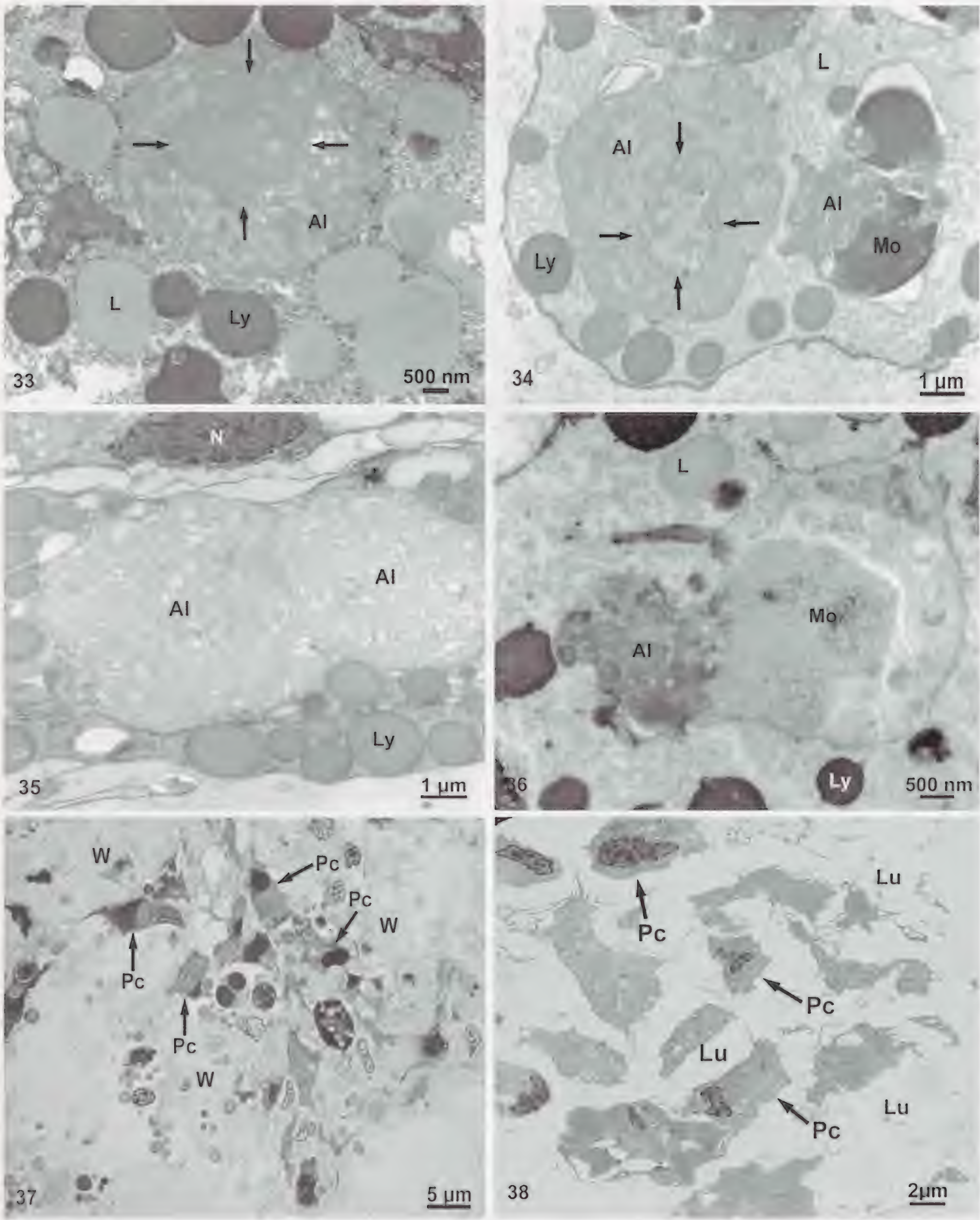
Sertoli cells typically contained structures designated as amorphous inclusions (Al). These bodies were of variable diameter and number in Sertoli cells (Figs. 15, 20, 24). At the ultrastructural level, an apparent metamorphosis of the internal structure of the inclusions was observed. Some Al displayed more distinct organelles such as mitochondria, rough endoplasmic reticulum and nuclei (Fig. 32) while others contained material reminiscent of a nucleus (Figs. 33, 34) or were primarily composed of amorphous material (Fig. 35). Lipids and lysosomes were observed in close contact with the Al (Figs. 33–35) and some Al appeared in close contact with each other (Fig. 35). Apparent transitional stages between morulae and Al were observed (Figs. 34, 36). The Al stained a yellowish brown color with Feulgen's stain (Fig. 24) and they had a continuous outer membrane (Figs. 33, 34).

Phagocytes

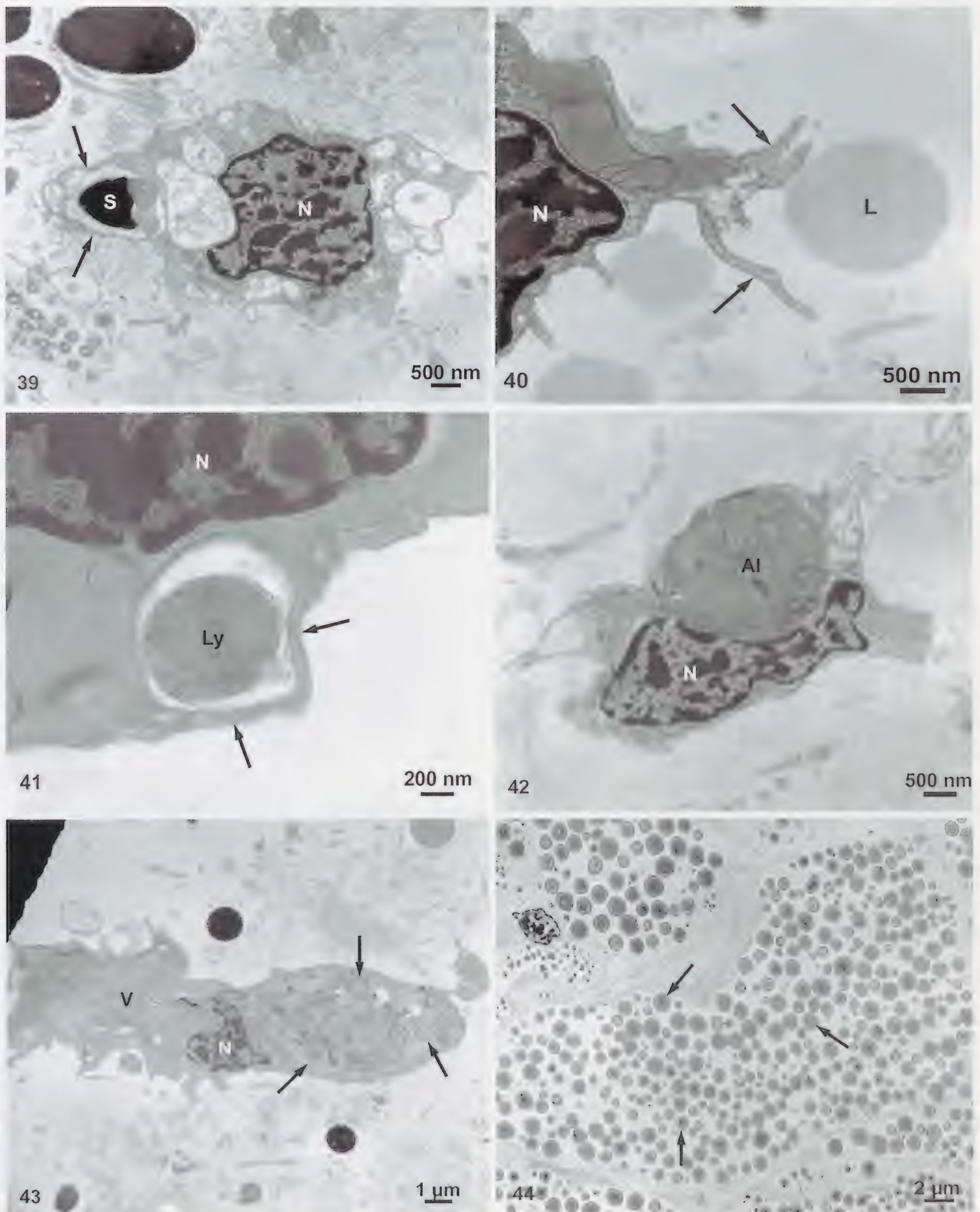
Cells recognized as phagocytes, based on similarities to the morphology and engulfing activity of cells from molluscs and other inver-



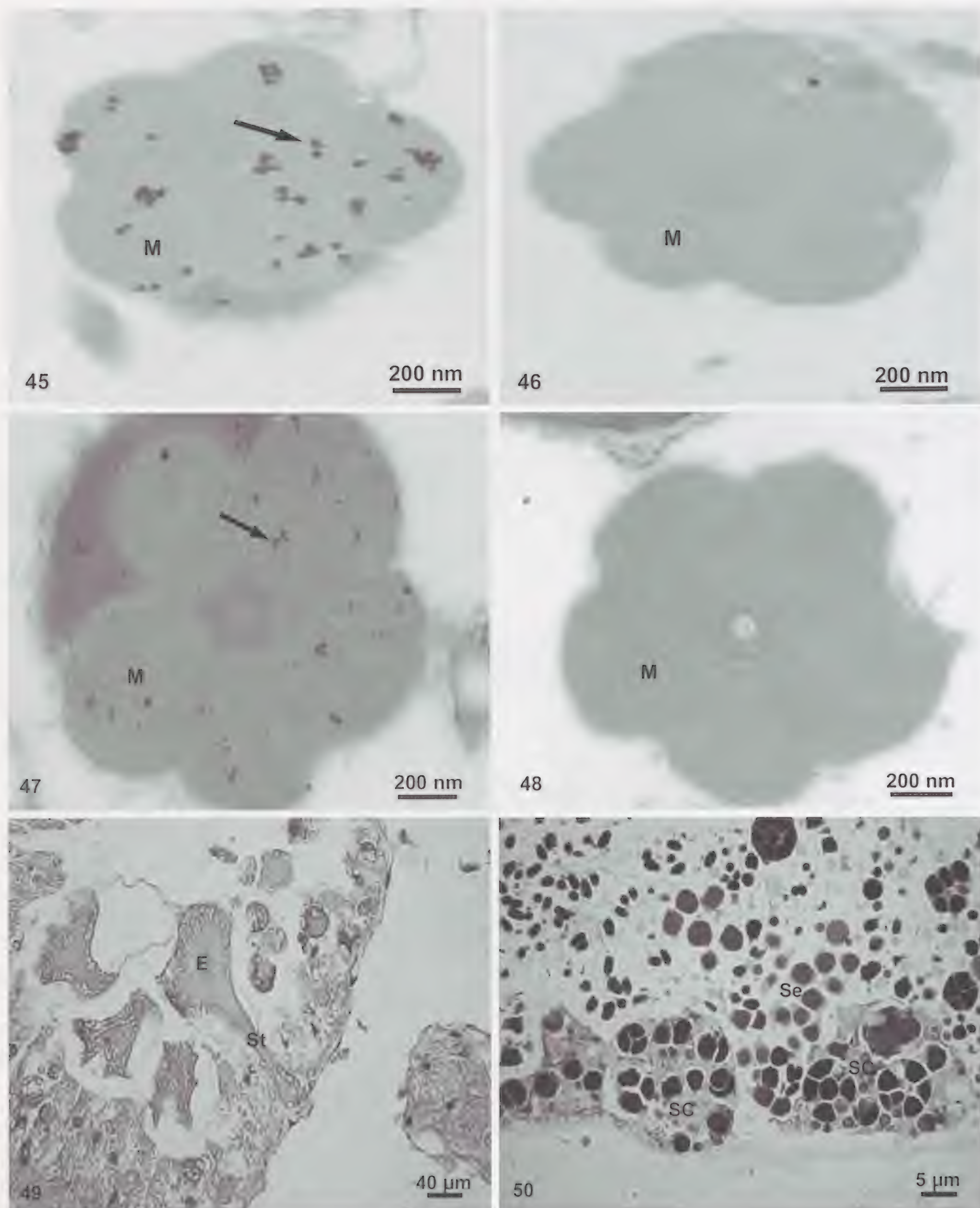
FIGS. 26–32. Atypical spermatogenic pathway. FIG. 26: Partial atypical pathway Sertoli cell containing spermatozoa clusters; FIG. 27: Atypical pathway spermatozoon showing how its flagellum wrapped around the spermatozoon; note that the spermatozoon is in cross section at the nuclear level; FIG. 28: Atypical pathway spermatozoon with bullet shaped nucleus; note flagellum fragment wrapped around spermatozoon; FIG. 29: Atypical pathway spermatozoon showing five mitochondria in cross section of midpiece; FIG. 30: Atypical spermatozoa axonemes in cross section; note two central microtubules (arrows); FIG. 31: Cell organelles and spermatozoa scattered in an acinus lumen as the Sertoli cells broke down in the atypical pathway; FIG. 32: Apparent early stage amorphous inclusion in Sertoli cell of atypical pathway; note the presence cellular organelles.



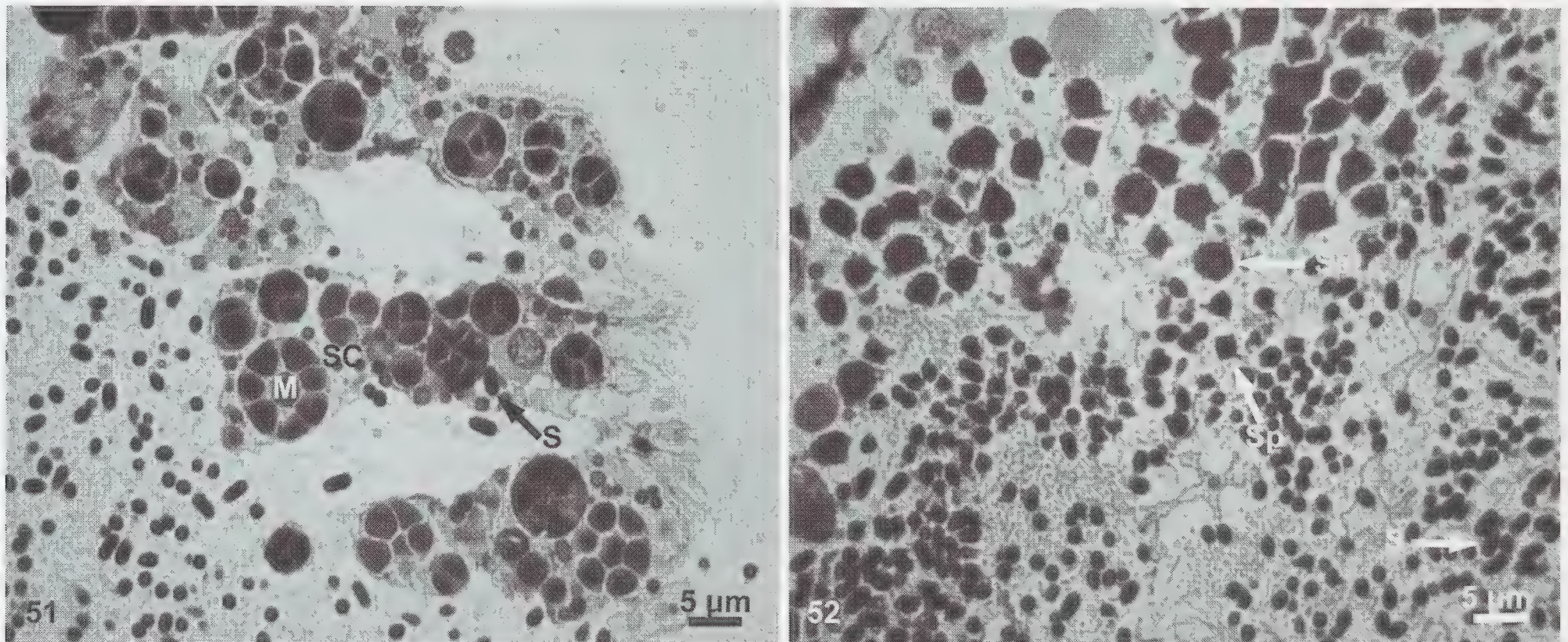
FIGS. 33–38. Atypical spermatogenic pathway cells and phagocytes common to both spermatogenic pathways. FIG. 33: Amorphous inclusion in Sertoli cell of atypical pathway; note the presence of nuclear-like material (arrows); FIG. 34: Apparent early breakdown stages of morulae into an amorphous inclusions; the amorphous inclusion on the right appears to be in an earlier stage of breakdown than the one on the left; note the presence of nuclear-like material (arrows); FIG. 35: Amorphous inclusions in close contact within Sertoli cell of atypical pathway; FIG. 36: Apparent transitional stage between morula and amorphous inclusion; FIG. 37: Phagocytes associated with an acinus wall; FIG. 38: Phagocytes associated with an acinus lumen.



FIGS. 39–44. Phagocytes and cells with calcium concretions common to both spermatogenic pathways. FIG. 39: Phagocyte engulfing a spermatozoon with its long, thin cytoplasmic extensions (arrows); note the nucleus with patches of condensed chromatin in its interior and lining its outer edge; FIG. 40: Phagocyte engulfing a lipid with its long, thin cytoplasmic extensions (arrows); FIG. 41: Lysosome engulfed by a phagocyte; the cytoplasmic extensions (arrows) encircle the lysosome; FIG. 42: Amorphous inclusion engulfed by a phagocyte; FIG. 43: Calcium concretions (arrows) engulfed by a phagocyte; vacuoles filled with electron dense material; FIG. 44: Calcium concretions (arrows) in cells nearby the male gonadal tissue.



FIGS. 45–50. Immunoelectron staining of July spermatozoa and concurrent presence of vitellogenic eggs and typical and atypical pathway cells in July. FIG. 45: Immunoelectron microscopy of July spermatozoon using the MCOX2e (M mt genome encoded protein) antibody showing positive presence (gold balls, arrows) of the protein on the inner mitochondrial membrane; FIG. 46: Control spermatozoon for Fig. 45; FIG. 47: Immunoelectron microscopy of July spermatozoon using the FORF (F mt genome encoded protein) antibody showing positive presence (gold balls, arrows) of the protein on the inner mitochondrial membrane; FIG. 48: Control spermatozoon for Fig. 47; FIG. 49: Vitellogenic egg, recognizable by its attached stock; FIG. 50: Presence of both typical and atypical pathway cells together in acinus in July.



FIGS. 51, 52. Atypical and typical spermatogenic pathway cells. FIG. 51: Atypical pathway Sertoli cell in acinus with spermatozoa in July; FIG. 52: Typical pathway cells with spermatozoa in acinus in July.

tebrates (but distinct from Sertoli cells), were noted in the male gonadal tissue from both the typical and atypical pathways. They were found associated both with the acinar walls (Fig. 37) and lumina (Fig. 38). These phagocytic cells were roughly rectangular in general outline and each contained a large nucleus with patches of condensed chromatin in its interior and lining the nuclear periphery (Figs. 38–43). Long, thin cytoplasmic extensions were observed enclosing a spermatozoon (Fig. 39), lipid droplet (Fig. 40), lysosome (Fig. 41), an AI (Fig. 42) and calcium concretions (Fig. 43). The calcium concretions were the only phagocytized material not observed in cells from the typical or atypical pathway. Concretions were seen in large quantities in cells nearby the male gonadal tissue (Fig. 44).

Immunoelectron Microscopy of Spermatozoa

Using single antibody experiments, some spermatozoa mitochondria were shown to possess the MCOX2 protein, encoded by the M mt genome, in their inner mitochondrial membranes (Figs. 45, 46), whereas the mitochondria from some other spermatozoa were shown to possess the FORF protein, encoded by the F mt genome, in their inner mitochondrial membranes (Figs. 47, 48).

Overlapping Presence of Vitellogenic Eggs, Typical and Atypical Spermatozoa

Vitellogenic eggs, recognizable by their attached stalks (Fig. 49), were observed from May through October. Both typical and atypical

spermatogenesis occurred simultaneously during this same time period (Figs. 50–52). Figures 50, 51, and 52 are from samples taken in July when fertilization takes place in this particular population of *V. ellipsiformis* (Chakrabarti et al., 2007, 2009).

DISCUSSION

In *Venustaconcha ellipsiformis*, spermatogenesis occurs along two distinct processes called the typical and atypical pathways. Spermatozoa produced by the two pathways were morphologically identical with a single acrosomal vesicle. While spermatozoa ultrastructure has been used to estimate phylogenetic relationships in bivalves, it has been suggested that acrosome structure should also be evaluated in relation to the amount of egg jelly necessary to dissolve prior to spermatozoon penetration. For example, brooding bivalves tend to exhibit less egg jelly presumably because the eggs are protected from environmental stresses and the univesicular acrosome likely provides adequate enzymes to dissolve the relatively small amount of jelly (Popham, 1979). *Venustaconcha ellipsiformis* is a long term brooding species (bradytictic) (van der Schalie & van der Schalie, 1963) and, as Popham (1979) noted, the structure of the acrosome in a species such as this could be more of a response to the functional aspects of the fertilization events noted above and thus have limited phylogenetic significance.

The developmental origin of morulae has long been an enigma as has that of atypical sperma-

tozoa. No previous study in which morulae were reported (Heard, 1975; Kotrla, 1988, 1989; Haggerty et al., 1995; Byrne, 1998; Matos et al., 1998; Garner et al., 1999; Shiver, 2002; Cek & Sereflisan, 2006; Chatchavalvanich et al., 2006) has documented a source for the morulae. As Dohman (1983) pointed out, some studies of gastropod mollusks, such as *Epitonium tinctum*, *Bithynia tentaculata* and *Crepidula plana*, have indicated that spermatogonial cells were considered the source of atypical spermatozoa, but these atypical spermatozoa did not involve morulae and the resulting spermatozoa were not morphologically indistinguishable from typical spermatozoa as is the case in *V. ellipsiformis*. As stated previously, we propose that the origin of the morulae, and thus of the atypical pathway, is due to spermatogonial cells located within Sertoli cells. Thus, there appears to be a developmental connection between the typical and atypical pathways via the spermatogonial cells. The atypical pathway spermatogonial cells (2N) were indeed seen dividing into groups of spermatogonial cells that were enclosed within the Sertoli cells and the 1-celled morulae would be the analog of the primary spermatocytes (2N) of the typical pathway. As the spermatogonial cells continue to divide, 2, 4, 8, 16 and 32-celled morulae are produced. There are no cells that are morphologically similar to the secondary spermatocytes of the typical pathway in this scheme, but that does not preclude the possibility that some of the cells in morulae are analogues of secondary spermatocytes but with different morphologies. One possibility is that the cells in the morulae would initially undergo mitotic and subsequently meiotic divisions ultimately producing 32-celled morulae wherein each cell would mature into a haploid atypical spermatozoa. Alternatively, a hypothesis that the atypical pathway represents ameiotic spermatogenesis (Kotrla, 1989), producing diploid spermatozoa, cannot be ruled out at present. The future use of densitometric and/or fluorometric methods (e.g., Hardie et al., 2002) to estimate spermatozoon ploidy level would facilitate the evaluation of these two hypotheses. Nevertheless, in *V. ellipsiformis*, we interpret the morulae as containing 1, 2, 4, and the presumed 8, 16, or 32 cells as providing a strong inference that the cells in the morulae were dividing. A careful examination of Figure 1A in Matos et al. (1998) reveals that a similar interpretation is also justified for that study. Apparent stages of division were noted in *V. ellipsiformis* as was decondensed nuclear material typical of dividing cells. It ap-

peared that cell division in morulae was not necessarily synchronous among the cells, as some cells were observed dividing when others were not, and mature spermatozoa were noted among cells within the same morula. Aggregates of atypical spermatozoa in membrane bound sacs (i.e., the membrane that previously surrounded a single morula) resulted within the Sertoli cells, and these spermatozoa eventually were released into the acinar lumen.

The observational evidence and logical reasoning for this hypothesis regarding the origin of the atypical spermatozoa is compelling. First, the spermatozoa must be generated from germ cells and the spermatogonial cells are the only known cells of this type available. Further, no observations were made of phagocytic activity by Sertoli cells, which could have led to the uptake of spermatids from outside the Sertoli cells. The possibility that spermatids from the typical pathway remained in acini after October and continued to form morulae until the following May also seems remote. Therefore, there is no evidence that the origin of morulae cells was from the typical pathway spermatids. There also is no evidence that the nuclei of the Sertoli cells were involved in the origin of the morulae cells. The Sertoli cell nuclei were never observed dividing in a manner similar to that observed in typical pathway spermatogonial cells. It is also notable that Sertoli cells apparently transitioned from containing many to few or no spermatogonial cells (Figs. 18, 19), which supports the contention that spermatogonial cells did continue to divide to create the morulae cells. Further, spermatozoa from the atypical pathway were initially observed inside membrane bound sacs in clusters which would be a logical expectation if they are developing from morulae cells also found inside a membrane. If further studies confirm the hypothesized transition of morulae cells from diploid to haploid, the morulae cells that are all currently referred to as spermatids may need to be divided into two groups with names referring to the diploid and haploid states such as "D morulae cells" and "H morulae cells". The last set of haploid morulae cells prior to their maturation into atypical spermatozoa would be the true spermatids.

Chatchavalvanich et al. (2006), in a light microscopy study, also observed morulae in the unionoid bivalve *Hyriopsis bialatus* that were essentially identical to those found in *V. ellipsiformis*. Chatchavalvanich et al. (2006) proposed a similar atypical pathway of spermatozoa production via morulae for *H. bialatus* as

we do for *V. ellipsiformis*. However, they called the original one-celled morula a spermatogonial cell and concluded that the cell types in the developing morulae included primary and secondary spermatocytes along with spermatids and spermatozoa. The morulae cells in the Chatchavalvanich et al. light micrographs appear remarkably similar to those observed at the light microscopy level in the present study. With the added benefits of electron microscopy in our study, however, it is clear that the cells within the morulae do not share characteristics with spermatogonial cells or primary and secondary spermatocytes but rather are morphologically very similar to spermatids. We suggest that the same is true of the morulae cells in Chatchavalvanich et al. (2006). With this difference in interpretation noted, however, we are in agreement with Chatchavalvanich et al. (2006) that spermatogonial cells are the likely progenitors of atypical spermatozoa and that atypical spermatozoa develop from spermatid cells in morulae. The presence of spermatozoa within the morula in Figure 23 provides substantial evidence that morulae cells do undergo development into spermatozoa, which is in agreement with conclusions and inferences from other studies (Heard, 1975; Kotrla, 1988; Haggerty et al., 1995; Matos et al., 1998; Garner et al., 1999; Shiver, 2002; Cek & Sereflisan, 2006; Chatchavalvanich et al., 2006).

Morulae consisting initially of spermatid-like cells were found in high numbers in Sertoli cells of the atypical spermatogenic pathway in *V. ellipsiformis*. Similar structures have been reported in other unionoid bivalve species (see Introduction). Shiver (2002) noted that morulae cells of *Lampsilis rafinesqueana* were precursors of spermatids and spermatozoa, while Jones et al. (1986) and Bloomer (1946) inferred the same for *Cucumerunio novaehollandiae* and *Anodonta cygnea*, respectively. Those depicted in *Prisodon alatus* (Matos et al., 1998) closely resemble the morulae observed in *V. ellipsiformis*. Matos et al. (1998) suggested that the Sertoli cells were somehow absorbing spermatids produced by a “natural spermatogenesis” and grouping them together inside a membrane. Further, Matos et al. (1998) hypothesized that microtubules in pseudopodia moved the morulae within the Sertoli cell and the spermatids then matured into spermatozoa. Matos et al. (1998) do not speculate on the specific origin of the spermatids or the function of the resulting spermatozoa and it is not clear what is meant by “natural spermatogenesis”.

However, no pseudopodia were seen on the Sertoli cells of *V. ellipsiformis* and no engulfment of any type of material by Sertoli cells was observed. It is possible that the collections of spermatozoa in *V. ellipsiformis* represented phagocytized spermatozoa as was seen in the study of sea urchins (Reunov et al., 2004). However, as with the morulae, no engulfment or digestion of spermatozoa by Sertoli cells of *V. ellipsiformis* was observed similar to the phagocytic activity of the NP cells in the Reunov et al. (2004) report. On the contrary, in *V. ellipsiformis*, normal appearing spermatozoa were released into the acinar lumen during atypical spermatogenesis. Also, it seems unlikely that flagella would be carefully wound around the nucleus and mid-piece of spermatozoa during a phagocytic or engulfment event. Based upon the above evidence, it is concluded that Sertoli cells in *V. ellipsiformis* are not involved in phagocytosis of spermatids that form the morulae or spermatozoa in Sertoli cells.

Sertoli cells have been observed involved with phagocytic activity during spermatogenesis in many other molluscs (Syasina, 1992), but they were not found so associated in *V. ellipsiformis*. Because *V. ellipsiformis* male acini were cleared of debris, a search was made to locate alternative cells that could be performing this important process instead of Sertoli cells. There are other cells in the male acini, distinct from the Sertoli cells, which we interpret as phagocytes. Their general appearance was quite different from the Sertoli cells (Figs. 15, 19 vs. Figs. 37, 38). These presumed phagocytic cells also demonstrated the classic stages of phagocytosis, for example, endocytosis of cellular debris using long cytoplasmic extensions and apparently containing digested cellular materials and organelles. No cells resembling hemocytes (Dundee, 1953; Higashi, 1980; Wen et al., 1994; Burkhard et al., 2009), which also can function as phagocytes, were seen in the acini of *V. ellipsiformis*.

While there are atypical spermatogenic pathways in a variety of organisms (Cook & Wedell, 1999; Ferraguti et al., 2002; Till-Bottraud et al., 2005; Paccagnini et al., 2006; Immler, 2008), including molluscs (Buckland-Nicks & Chia, 1986; Jespersen et al., 2001; Buckland-Nicks & Thompkins, 2005), a pathway including AI in Sertoli cells has not been reported. The role of Sertoli cells in destruction of materials by lysosomal activity is well documented in molluscs (Buckland-Nicks & Chia, 1986; Pipe, 1987; Dorange & LePennec, 1989; Sousa et al., 1989;

Rocha & Alzevedo, 1990; Syasina, 1992; Eckelbarger & Davis, 1996; Kiyomoto et al., 1996; Erkan & Sousa, 2002). It is possible that, in *V. ellipsiformis*, the AIs are organelles resulting from lysosomal activity, and the observed transition of the appearance of the material inside them occurs as sperm morulae break down into or are digested by the AIs. Under this scenario, the peculiar yellowish brown color (using the Feulgen stain) of the AIs could be indicative of digesting DNA from the morulae cells. Apparent steps of morulae undergoing a breakdown process were observed many times. Figures 32–36 are particularly suggestive of various phases of morulae digestion, especially when comparing Figure 21, an intact morula, to Figures 33 and 34, where morulae appear to be undergoing the digestion process. The close proximity of sperm morulae and AIs with lipids could indicate that the lipids provide energy for this process, while the nearby lysosomes could contribute enzymes for breaking down the morulae. Under this interpretation, AIs could be considered a type of secondary lysosome. It is also possible that, as the morulae degenerate into AIs, the individual AIs combine together. This would mean that the AIs were undergoing a fusion event as is typical of lysosomes (Karp, 1999) and is supported by the observed close special association between AIs.

Phagocytic and lysosomal activities have been reported as important in clearing out the acini of unused spermatozoa in preparation for upcoming spermatogenesis during the yearly reproduction cycles in sea urchins (which are deuterostomes and thus distantly related to bivalves) (Reunov et al., 2004). Reunov et al. (2004) observed accessory cells called nutritive phagocytes (NPs) that engulfed spermatozoa. The individually phagocytized spermatozoa then aggregated into larger heterophagosomes, which were digested into bodies referred to as remnant bodies. Remnant bodies were eventually destroyed, leaving a vacuolar space that disappeared through cytoplasmic compression. The remnant bodies are morphologically very similar to the AIs of the present study and appear to be formed by a similar process involving lysosomal activity. However, there are some important differences between the formation of the remnant bodies in sea urchins and the AIs in *V. ellipsiformis*. First, the AIs could be a product of morulae digestion whereas the remnant bodies were involved in spermatozoa digestion. Second, the spermatozoa were phagocytized while

there was no evidence of a similar process with the morulae, that is, no long, thin extensions commonly associated with phagocytes were observed on Sertoli cells. Finally, there was no evidence of AI destruction and reabsorption within the cells. Rather, the AIs were released intact into the acinar lumen as the Sertoli cells broke down and some AIs were seen being absorbed by phagocytes that were structurally distinct from the Sertoli cells. If the AIs in *V. ellipsiformis* are digested morulae, then a substantial percentage of sperm morulae created in the atypical pathway are broken down. Perhaps the contents of some of the morulae cells are recycled as nutrients within the Sertoli cells. A similar role of providing nutrients to developing spermatozoa has been reported for Sertoli cells in other molluscs (Pipe, 1987; Dorange & LePennec, 1989; Syasina, 1992). Alternatively, some morulae could be defective and thus could be selected for destruction as occurs in triploid mussel spermatocytes that do not mature (Kiyomoto et al., 1996).

The typical and atypical spermatogenic pathways overlapped in *V. ellipsiformis* from May to October. Because the spermatozoa produced by the two distinct pathways were morphologically indistinguishable, it was impossible to tell which pathway produced them unless the spermatozoa were located inside the clusters of the atypical pathway. It is important to note that vitellogenic eggs were also present in this same May to October time frame. At present, the only documented fertilization event for this species in Michigan occurs in late July. It is possible that additional fertilization events occur in October, for example, in more southern *V. ellipsiformis* populations, but not in Michigan due to the colder fall temperatures. Nevertheless, the atypical pathway might be active year round because it requires less energy and can withstand the colder temperatures of fall and winter or because it has its more of its own energy source in the recycling of nutrients via the breakdown of the morulae into AI which then act as an energy reservoir. This hypothesis requires that atypical spermatozoa have a relatively long functional longevity within testes.

There are several hypotheses that could account for the two spermatogenic pathways in unionoid bivalves. One is that only spermatozoa from the typical pathway are responsible for fertilization and the atypical spermatozoa have some other role. The positive stain for DNA in atypical spermatozoa rejects the hypothesis that they are apyrene, that is, devoid of DNA,

as is the case with the atypical spermatozoa of many organisms. This further supports the hypothesis that atypical spermatozoa can fertilize eggs. The morphological similarity between the typical and atypical spermatozoa also lends credence to this hypothesis, as does the presence of the dual spermatogenic pathways in all unionoid families (Heard, 1975, unpublished; Kotrla, 1989). The latter point suggests that these pathways were both operative in the unionoid common ancestor (> 200 million years ago) and that they both persisted to the present in all familial lineages. Overall, the above evidence argues strongly for the fertilization functionality of atypical spermatozoa in unionoids while rejecting a nonfunctional hypothesis.

Given the above evidence supporting the fertilization functionality of atypical spermatozoa, the pivotal question remains as to why there are two spermatogenic pathways in *V. ellipsiformis* and in unionoids generally. One hypothesis is that production of atypical spermatozoa during times of the year when there is no fertilization (November to early May) is a means to keep the germ tissues active at a low level so that they can be readily reactivated at a high rate when conditions are appropriate for fertilization. This hypothesis implies that atypical spermatozoa are not used for fertilization. However, this hypothesis can be rejected by the observations that atypical and typical spermatogenesis occur simultaneously in *V. ellipsiformis*. Furthermore, the long term maintenance of ultrastructural identity between these two pathways' end products would not be expected due to relaxed selection on the atypical pathway (see below).

A second hypothesis is that the spermatozoa from one of the two pathways play only a structural role in the formation of the spermatzeugmata (the spherical aggregates of spermatozoa released by unionoid males; Lynn 1994) while the other pathway's spermatozoa are responsible for actually fertilizing the eggs. However, if the spermatozoa from one pathway are only structural, why would natural selection maintain the presence of DNA, acrosomes and identical ultrastructure in the "structural" spermatozoa for > 200 million years? Rather, under the structural hypothesis, it would be expected that unionoid spermatozoa produced by the "structural" pathway would have attained the apyrene condition after > 200 million years of phylogenesis.

A third hypothesis for the long-term maintenance of two spermatogenic pathways is

that atypical spermatozoa are produced and released prior to the production and release of typical spermatozoa in order to communicate with females to facilitate synchronized gamete maturation, and that only typical spermatozoa participate in fertilization. Under this hypothesis, one would predict that (1) atypical spermatozoa would always be produced/released before the typical spermatozoa and (2) natural selection would eventually reduce the energetic expenditure on atypical (i.e., non-fertilizing) spermatozoa by eliminating the acrosome, DNA, etc. However, given the synchronized occurrence of typical and atypical spermatogenesis in *V. ellipsiformis* and the long term ultrastructural identity of these two pathways' end products, the "communication" hypothesis can be rejected.

A fourth hypothesis is that the two distinct pathways' spermatozoa are involved in the maintenance of dioecy (i.e., separate sexes) and 50 : 50 sex ratios in populations of unionoid bivalves. Heard (1975) suggested that the atypical pathway in *Utterbackia imbecillis* (always hermaphroditic; W. R. H., personal observation [but see Heard, 1975]) is greatly reduced relative to the other anodontine species (all dioecious) he examined. This observation is consistent with the hypothesis that both spermatogenic pathways are required for the maintenance of dioecy and 50 : 50 sex ratios in unionoids. The IEM-based inference reported herein, that a spermatozoon contains either F- or M-genome-bearing mitochondria, also corroborates this hypothesis due to the recent discovery that the genetic transmission of both F and M mt genomes is required for the maintenance of dioecy in unionoids, i.e., with M mt genomes responsible for maleness (Breton et al., 2011). Thus, the above observations (1) reject the hypothesis that only M mt genome-bearing spermatozoa effect fertilization in species with DUI (Venetis et al., 2006) and (2) are consistent with the hypothesis that the dual spermatogenic pathways in unionoids are a specialization to produce two types of spermatozoa, that is, those with M genome-containing mitochondria and those with F genome-containing mitochondria. If, as proposed earlier (Breton et al., 2011), the F and M mt genomes directly participate, through interactions with the nuclear genome, in the genetic regulatory network specifying dioecy in unionoid bivalves, the inference can be made that spermatozoa with F genome-containing mitochondria are less likely to facilitate maleness and could thus participate in

the maintenance of 50 : 50 sex ratios in bivalve populations. In other words, it is hypothesized that males in male-biased populations produce a larger proportion of spermatozoa with F genome-containing mitochondria than males in female-biased populations. Because the presence of both M mt genomes and the atypical spermatogenic pathway are derived character states, it is further hypothesized that the atypical pathway produces spermatozoa with M genomes while the typical pathway produces spermatozoa with F genomes. Nevertheless, the long-term co-occurrence of two highly unusual phenomena in unionoid bivalves, that is, (1) dual spermatogenic pathways yielding morphologically identical spermatozoa and (2) high fidelity DUI (Hoeh et al., 2002), strongly suggests that the two phenomena are causally linked (Zouros, 2000). Further research is required to test the “maintenance of dioecy and 50 : 50 sex ratios” hypothesis and, if it is confirmed, determine the mechanisms underlying these two unusual phenomena.

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THE MALACOLOGICAL WORKS AND TAXA OF SYLVANUS HANLEY (1819–1899)

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ABSTRACT

The malacological works of Sylvanus Hanley (and his relative Charles Thorpe) are discussed and their dates in some cases clarified. The taxa that first appear in these works are listed, their type specimens noted when known, and the current status of the available taxa discussed. Of the 375 species-group names that first appear in these works, 367 are available, and 8 are *nomina nuda*. Of the 367 available species-group taxa, approximately 219 are now considered valid. Approximately 60% of the available species are represented by type material, mainly in the Natural History Museum in London and in the Leeds City Museum in Leeds, U.K. Hanley also described several genus-group and family-group names, some of which are still considered valid.

Key words: Sylvanus Hanley, Charles Thorpe, bibliography, taxonomy.

INTRODUCTION

A decade ago, Norris & Dance (2002) authored a biography of Sylvanus Hanley, a significant British malacologist who published from the 1840s to the 1880s. He was particularly noted for his contributions on the Bivalvia and on the non-marine fauna of India and adjacent regions. In a review of that paper (Coan & Kabat, 2002), we noted that this otherwise excellent biography also included a bibliography of Hanley's papers and books that had some dating errors, as did its list of Hanley's species, which omitted a number of taxa.

In 2002, we intended to prepare an improved bibliography and a more complete, analytical list of Hanley's taxa and type material. However, the press of other projects and the difficulty of access to some scarce literature postponed that project. Now that almost all of Hanley's works and more of the relevant secondary literature are available online, primarily in the Biodiversity Heritage Library, we have returned to that project, resulting in the present paper.

MATERIALS AND METHODS

† We reviewed all of Hanley's publications, indexed the new taxa, and reviewed the secondary literature to identify subsequent references

to Hanley's publications and taxa. To ensure that our coverage of Hanley's taxa was as thorough as possible, we reviewed all the taxa attributed to him or to Charles Thorpe in the online, searchable versions of Sherborn (1922–1933), Neave et al. (1939–1996) and Ruhoff (1980). One of us (Kabat) spent one week at the Natural History Museum of the United Kingdom (London) (25–29 June 2012) and two days at the Leeds City Museum (2–3 July 2012), reviewing Hanley type specimens, both those already catalogued as types and those hitherto unrecognized in the general collection. The Hanley type material in London has been frequently studied by the curatorial staff and outside researchers over the past five decades. In contrast, the Hanley type material in Leeds has been only partially curated and remains largely overlooked by outside researchers.

The measurements of type specimens, when given herein, were made with digital (electronic) calipers. The measurements of gastropods are height (parallel to the shell axis) and width (perpendicular to the shell axis, at the widest part of the aperture), except for the one low-spined *Haliotis*, for which the length and width are given. The measurements of bivalves are length (parallel to the hinge line) and height (perpendicular to the length and running through the uppermost point of the hinge line). For most bivalves, the length corresponds to

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the maximum dimension; for some bivalves with trapezoidal shapes or otherwise diagonally elongated valves, the length may be slightly shorter (by 5% to 10%) than the maximum dimension.

Hanley's Life and Malacological Research

Hanley was born in 1819 in Oxford and studied at Oxford University, obtaining a B. A. degree in 1841. He soon inherited sufficient funds from his father, so that he did not have to work for a living, and devoted the rest of his long life (he died at age 80 in Penzance, Cornwall) to the study of mollusks. Adrian Norris has maintained an interest in Hanley and his family, and he intends to publish more on that subject (A. Norris, pers. comm., 15 November 2011).

Hanley was a contemporary of the shell collector Hugh Cuming (1791–1865), and described many species from the Cuming collection, as did his fellow conchologists Lovell Reeve (1814–1865), George Brettingham Sowerby I (1788–1854), and George Brettingham Sowerby II (1812–1884). For many of his new species (other than the Tellinidae, as noted below), Hanley was comparable to Reeve and the Sowerbys when it came to describing new species – all four did not hesitate to describe as new species what are now considered to be morphological or color forms of previously described taxa, because they did not recognize the significance of variation within a species. Hanley also described a number of “varieties” (equivalent to subspecies), most also based on unique specimens and mere color forms. Nor did Hanley and his contemporaries refrain from describing new species based on material from unknown or uncertain localities, or based upon unique specimens. When it came to molluscan genera, they preferred to use the Lamarckian genera in their broadest sense, with all the species in any given family being assigned a single genus.

Hanley's first book, at the age of 21 and while still in college, was the appropriately titled *The young conchologist's book of species* (Hanley, 1840), which had descriptions of some 600 species, three newly described by Hanley, all now synonymized. This book soon went into a second edition (Hanley, 1842a), which included

a glossary and index. Tomlin (1945: 91) briefly discussed these editions. Hanley (1842: 154) stated that the “localities and authorities [in the index] are chiefly extracted from Dr. Jay's *Catalogue*,” referring to the *Catalogue of Recent Shells* by John C. Jay (1808–1891), which went through three editions in the 1830s (for example, Jay, 1836).

Hanley's second book was the “second” (actually third) edition of William Swainson's *Exotic conchology* (Hanley, 1841). Hanley's edition has little scientific value and has only one new taxon, *Cochlycopa*, in the Volutidae. Hanley (1841: 4) explained that: “Mr. Swainson on quitting England [for New Zealand] having left this beautiful work in an unfinished condition, to me has been committed the task of reducing the whole into systematic arrangement, of drawing up descriptions of species, and adding such synonyms as ... require[d].” Abbott (1968: xxiv), in reprinting this work, noted that: “An examination of several copies of Hanley's edition indicates that he probably had left-over copies of some of Swainson's 1834 plates. Both Swainson's and Hanley's editions were hand-painted over the lithographed line drawings, so that no two copies were identical.” Concerning Swainson's two editions, see Sherborn & Reynell (1915) and McMillan (1963).

Hanley then focused his attention on the Bivalvia, publishing the first edition (or version) of his *Illustrated, enlarged and English edition of Lamarck's species of shells* (Hanley, 1842–1843), which, despite its title, was limited to the Bivalvia. Evidently not satisfied with this work, in 1843, Hanley reissued it with some additional text and plates, under a slightly revised title, *An illustrated and descriptive catalogue of Recent shells* (Hanley, 1842–1846). Hanley then reissued it yet again in 1856, with further new text and plates, under yet a third title, *An illustrated and descriptive catalogue of Recent bivalve shells* (Hanley, 1842–1856). We discuss the problems in dating these publications in the next section, but they are of great significance in containing the descriptions and illustrations of many new species, as well as re-descriptions of those of earlier authors. It must be emphasized that – in contrast to most of Hanley's illustrated publications, in which the specimens are usually illustrated at life-size – these books on bivalves

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FIG. 1. Plate 2 from *An illustrated, enlarged, and English edition of Lamarck's species of shells* (Hanley, 1842–1843). Note that the specimens are all printed at the same size, regardless of their original dimensions. Plates 1–3 of the first edition were not reprinted in the later editions or versions.



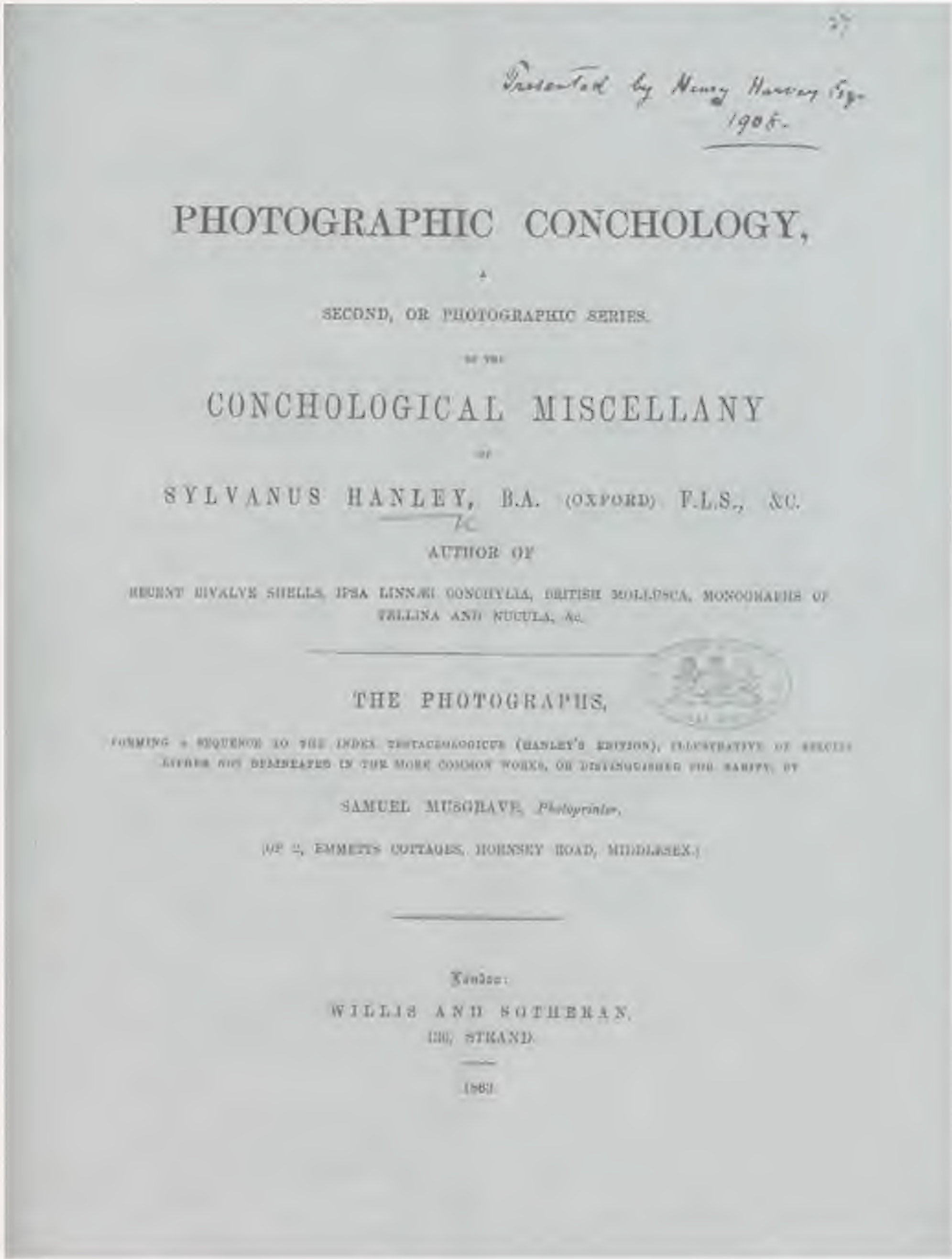


FIG. 2. Title page, *The Photographic Conchology* (Hanley, 1863). The first book to use photography for illustrating mollusks, with all the black-and-white photographs completely overpainted in color. Thus, nothing remains visible of the original photograph except for the outline of the shells.

have all the illustrated specimens enlarged or reduced to approximately the same size. While this makes for neatly ordered plates (Fig. 1), it makes it harder to match potential type specimens with the illustrations. This work, and Hanley's later *Index testaceologicus* (Hanley, 1856b), were assembled in part on the model of the *Index testaceologicus* (1818, 1823–1825, 1828a, b) by William Wood (1774–1857). In contrast to Wood's work, which used symbols to indicate whether a figure was reduced, life-size, or enlarged, Hanley generally did not provide this information on his plates in these works. Hanley's personal copy of this work (Hanley, 1842–1856) is now in the Library archives at the Academy of Natural Sciences of Drexel University (Philadelphia). It contains interleaved blank pages with annotations about generic allocations and specimens in his collection. It also has many additional pages onto which he pasted illustrations of shells from other sources (Callomon, 2010).

Perhaps Hanley's most important book at the time was *A history of British Mollusca, and their shells* (Forbes & Hanley, 1848–1853), published in four volumes, and co-authored with the distinguished marine biologist Edward Forbes (1815–1854). This work is noteworthy for its extensive anatomical descriptions and ecological observations of the included species, which may lead some to conclude that the “biological” component must have been entirely the work of Forbes, for Hanley's other papers contain no information on anatomy or ecology. However, Norris & Dance (2002: 367) suggested that “there is evidence throughout the work that Hanley contributed more than his fair share” and noted that many of the specimens were collected by Hanley “around the English coast,” including with a dredge. Charles Darwin was among the numerous researchers who used this reference, as revealed by his correspondence with the paleontologist Charles Lyell (C. Lyell to C. Darwin, 1 May 1856; C. Darwin to C. Lyell, 14 Oct. 1862, online at <http://www.darwinproject.ac.uk>). Darwin also asked the botanist J. D. Hooker whether he had a copy of this “grand work” that Darwin could examine (C. Darwin to J. D. Hooker, 14 Nov. 1857). Darwin, in his monographs on the barnacles, acknowledged Hanley for having provided him with an important reference on barnacles.

Hanley's next book was *The conchological miscellany* (Hanley, 1854–1858). In an afterword at the end of this book, Hanley explained that: “The plates of *Cyclostoma* (together with

those of *Melo*, *Pandora*, and *Amphidesma*) were originally intended to appear in the ‘Species Conchyliorum’ of the late G. B. Sowerby. He did not, however, publish them, but discontinued his beautiful, yet unremunerative, work, after the first number. Subsequently to his decease, a few imperfect sets were sold by one of the principal London dealers in second-hand books, and issued as the ‘Species Conchyliorum part ii’.” Petit (2009: 56–58) was unable to locate any copies so titled of this second part, the first part having been published by Sowerby in 1830.

Meanwhile, in the 1840s and early 1850s, Hanley was also studying the specimens in the shell collection of Linnaeus, which had been acquired by the Linnean Society of London through a purchase by James E. Smith (the specimens studied by Linnaeus in the Swedish royal collections remained in Uppsala, Sweden). Hanley's research led to two publications, a book entitled *Ipsa Linnaei conchyliæ* (Hanley, 1855) and an article, “On the Linnean manuscript of the ‘Museum Ulricæ’” (Hanley, 1859b). These remain important for their analysis of the molluscan species described by Linnaeus in the *Systema natura* and his other publications. Hanley made good use of the manuscripts of Daniel Solander (1733–1782) in analyzing the Linnaean species (Wilkins, 1955: 86–87). Unfortunately, Hanley apparently had a careless attitude towards the curation of the Linnaean collections in London; Norris & Dance (2002: 368) concluded that his curatorial efforts were so abysmal, mixing old and new specimens, that “it were better had he not started it,” although part of the blame may fall on subsequent curators of that collection prior to the 1950s.

Hanley then published a revised edition of Wood's *Index testaceologicus* (Hanley, 1856b). Norris & Dance (2002: 367) commended this work for its comprehensiveness and numerous illustrations, which they recognized was the product of “someone with an encyclopaedic knowledge of molluscan shells and their classification.” Coan & Petit (2011: 15) and Wilkins (1957: 157–158) further discussed this work.

Hanley's next book was the curiously published *Photographic conchology* (Hanley, 1863b), stated to be the “second, or photographic series” of the aforementioned *Conchological miscellany* (Fig. 2). The reader may be confused in examining this work, for all the illustrations are in vivid, almost garish color, yet color photography did not exist until

the 1940s. Hanley had black-and-white photographs of freshwater bivalves of the genera *Anodonta* and *Unio* (Unionidae) and *Cyrena* (Cyrenidae) completely over-painted in color, leaving no trace of the original black-and-white photographs. Norris & Dance (2002: 382, fig. 7) reprinted one of the plates, in color. Smith (1909: 196) noted that the "coloration [was] not always successful;" and the "method of production by photography secured correctness of outline, but the surface ornamentation is much obscured owing to reduction and the superimposed colour and gum-wash." In the 1850s and 1860s, this coloring of photographs was a popular technique (Henisch & Henisch, 1994), but Hanley never repeated the method. That said, this work does have the description of one new species, *Unio tirostris*, and the first illustrations of two previously described species of *Cyrena*. Some sources (Corns & Sparke, 1915: 102, 163) erroneously attributed authorship of this work to Samuel Musgrave, but he was the photographer, not the author of the text. Oddly, despite having published the first molluscan work based on photography, no image of Sylvanus Hanley is known.

Hanley's last malacological book was the *Conchologia Indica* (Hanley & Theobald, 1870–1876), co-authored with William Theobald (1829–1908). This book covered some of the non-marine molluscs of British India, including what is now Burma, Nepal, Pakistan, and Sri Lanka, with a few estuarine or brackish-water species included. Hanley never visited India, and the specimens in the book are largely those collected by Theobald and by William Henry Benson (1803–1870), both of whom did extensive collecting. Unfortunately, Hanley's careless attitude towards curation did much to ruin the scientific value of the collections of Benson and Theobald, who had carefully labeled their specimens with precise locality information. Norris & Dance (2002: 368) sadly noted that "Hanley acquired Benson's collections and manuscripts, replaced the original labels with ones of his own which merely read 'India,' and as if that were not enough, he lost Benson's precious manuscripts!" Naggs (1997: 56) forthrightly discussed Hanley's "unspeakable destruction of Benson's documentation" and quoted from a 1922 letter by H. H. Godwin-Austen, who knew Hanley, to Hugh Fulton, in which Godwin-Austen wrote that the Benson collection "was a splendid collection when it first went to Hanley. I remember it well and saw it just as Benson arranged it. He was most particular with locality. Hanley did not believe

in geographical distribution and soon removed it [locality data]." Nonetheless, students of the Indian molluscan fauna have to use the *Conchologia Indica*, because it has many new species of land snails from two biodiversity hot-spots – the Western Ghats (the mountainous region of southwestern India) and Sri Lanka (Naggs & Raheem, 2000; Raheem & Naggs, 2006; Raheem et al., 2009).

Hanley's last book was also his only non-malacological publication, the *Caliphs and sultans, being tales omitted in the usual editions of the Arabian Nights entertainments* (1868a; a second, seemingly identical edition was issued in 1870). This book, published by Reeve, is little remembered today, because Sir Richard Burton's edition of the *Arabian Nights* remains the definitive version. Kirby (1894: 268) tartly noted that Hanley's compilations "hardly require mention" as Hanley evidently did not do any original translations from the Arabic, but merely copied other sources.

Hanley also published his research in journals, primarily in the *Proceedings of the Zoological Society of London* (some articles were reprinted in the *Annals and Magazine of Natural History*). Most of his journal articles were isolated descriptions of new species; even when Hanley described several new species from the same family in a single article, he seldom compared the new species with each other, or with previously described taxa in that family.

Hanley authored several catalogues that attempted to list all the taxa known for a given family, including *Dolium* (now *Tonna*, in the Tonnidae), *Solarium* (now *Architectonica*, in the Architectonicidae), *Melania* (species now placed in a variety of genera of freshwater Cerithioidea), *Nucula* (Nuculidae, Nuculanidae and Yoldiidae) and *Tellina* (Tellinidae). Although a number of the new species described by Hanley in these catalogues have since been synonymized, and the *Melania* catalogue was promptly criticized by the Swiss malacologist Brot (1860), the catalogues were valuable at the time for their extensive listings of the described species in those genera. It should be noted that Hanley's monograph on the Tellinidae (1846a) was of higher quality than some of his other work, for a greater percentage of his new species in that family are still in use today – in contrast to the higher synonymization rates of Hanley's species in other molluscan families.

The Zoology Library of the Natural History Museum has a manuscript notebook volume ("*MS Note-Book on Conchology*" catalogued as

MSS HAN 1), evidently from the early 1850s, with Hanley's lengthy notes on several topics: (1) an index of species of *Melania*, followed by a shorter index of species of *Siphonaria*, presumably used to prepare his publications on those taxa; (2) notes on the Linnean shell collection, used for his 1855 *Ipsa*; and (3) notes on Wood's *Index testaceologicus*, accompanied by cut-and-pasted figures from Wood's edition of that book, evidently used by Hanley in preparing his 1856 edition.

J. B. S. Haldane (1892–1964), a strong advocate of evolutionary biology, was once asked by a churchwoman (or theologian; the sources vary) what the study of evolution had taught him about God. Haldane's famous and possibly misquoted reply was that "God has an inordinate fondness for beetles," referring to the remarkable biodiversity of that group. Williamson (1992) concluded that Haldane probably instead said: "the Creator, if He exists, has a special preference for beetles". It certainly can be said that "Hanley had an inordinate fondness for *Tellina*," since of the 375 species-group names established by Hanley, 94 (or 25%) were in the Tellinidae. Another seven families accounted for 113 species (30%), that is, Veneridae (25 species); Donacidae and Unionidae (18 species each); Architectonicidae (15 species); Mytilidae (14 species); Nuculanidae (12 species); and Ostreidae (11 species).

Bibliographic Notes on Some of Hanley's Works

Several of Hanley's books merit additional bibliographic discussion. One of these is the *British marine conchology* (1844). The title page of this volume gives the authorship as Charles Thorpe, and this was suggested by Norris & Dance (2002: 364, 375) to be a pseudonym of Hanley himself, given that this corresponds to Hanley's middle names. However, we think it was really by a relative (or friend of his parents) named Charles Thorpe, after whom Hanley could have been named. Why this volume was Thorpe's only published book or paper on mollusks remains a puzzle, but he is listed as a "collector" elsewhere (Forbes & Hanley, 1852: 203) and was also noted as a source of manuscript descriptions for some of the species descriptions in Hanley & Theobald's *Conchologia Indica* (1870–1876), in which his last name is spelled as both Thorp and Thorpe. The *British marine conchology* contains a Systematic List (pp. xvii–lx) by Hanley that also includes several new taxa. In this

list, Hanley specifically expressed doubt as to whether Thorpe's sole new species described in the book's main text, *Littorina patula* (p. 259), might instead be merely a variety of *L. rudis* (p. xxxix). That preface, in which Hanley commented on Thorpe's systematics, helps confirm that Hanley was not the author of the main text of this book.

The second work that merits particular attention is his monograph on bivalves, published in parts and under three different titles between 1842 and 1856. While it has been the subject of previous discussions and collations, such as that of Reynell (1918b), we have now clarified one key issue: most copies of the uncommon first version or edition, *An illustrated, enlarged, and English edition of Lamarck's species of shells* (1842–1843), included both an eight-page "Systematic List" and an eight-page "List of Illustrations" that covered as-yet unissued plates. In these lists, some new names first appear as *nomina nuda*. Later, when the plates containing the figures corresponding to the already printed names were produced in what became the second and third versions or editions of the book (1842–1846, 1842–1856), the species names became available, even though the main descriptive text for these taxa had not yet appeared. This recognition of the availability of several taxa from the previously issued plate captions, once the plates themselves were published, results in changes to the dates for a few bivalve taxa. Unambiguous use of dates and letters for each newly available part in the collation in Table 1 and in the Literature Cited herein serves to make clear the sequential appearances of the references and figures of each species, with an underlined date-letter in the catalog of Hanley's taxa, such as "1842d" indicating that point at which the name became available, if it was not made available at its first appearance in a plate caption alone.

Below we set forth the collation of this series. It should be noted that the second version or edition reprinted the first 224 text pages of the first version, along with the indexes; the third version or edition, in turn, reprinted the foregoing text from the first version or edition, along with the additional text pages 225–279 and plates 9–19 from the second version or edition. Oddly, the second and third versions or editions did *not* reprint plates 1–3 and the accompanying captions from the first version or edition. It should be noted that plates 4–8 were never published in any of the three versions or editions, and were not cited in the text.

TABLE 1. The sequential parts of the complex bivalve book by Hanley. *See Literature Cited for full titles & publication details.

Title*	Date range	Total Pagination/Plates	Pages/Plates	Date	Here as
[An illustrated, enlarged, and English edition of Lamarck’s species of shells, ...]	1842–1843	224 + 8 + [3] + 8 pp., 3 pls.	pp. 1–32, pls. 1, 2	late 1842	1842b
			pp. 33–144, pl. 3	early 1843	1843b
			pp. 145–224; 8 pp. (Systematic List), [3] pp. (List of Illustrations = explanations to pls. 1–3), 8 pp. (expls. to pls. 9–13)	late 1843	1843f
An illustrated and descriptive catalogue of Recent shells, ...	1842–1846	272 + 18 pp. everything above (except pls. 1–3 & their explanations)	pp. 225–272, pls. 9–13	late 1843	1843g
			pls. 14–16	1844	1844g
			pp. 9–18 (explanations to pls. 9–19), pls. 17–19	1846	1846c
An illustrated and descriptive catalogue of Recent bivalve shells.	1842–1856	xviii + 392 + 24 pp., pls. 9–24 – everything above (except pls. 1–3, their explanations & 8 pp. Systematic List)	pp. i–xviii, 273–392; pp. 19–24 (explanation for pls. 20–24), pls. 20–24	1856	1856a

We have also seen two versions of the first edition, *Lamarck’s species of shells*, the only difference noted being in the sizes of the type of the footnotes. Both the first and third editions have been digitized and are also present in hard copy in a number of natural history and university libraries. The second edition, however, is only known to us from a single copy in the Mollusca Library, Natural History Museum of the United Kingdom. Nissen (1967: 189) correctly listed the titles of all three editions, but the information that he provided on the pagination and number of plates in each edition is inconsistent with the copies that we have examined.

The third work that needs to be mentioned is Hanley & Theobald’s work on the fauna of British India (1870–1876). While a collation of this work has been available for many years (Prashad, 1927), it has been overlooked by some workers, who incorrectly dated all the included species as “1876.” Also, the authorship of the new taxa in this work is another source of confusion, because some of the new taxa are stated to be by “Hanley & Theobald,” while others are stated to be by “Hanley” alone. The latter taxa should be attributed to “Hanley, in Hanley & Theobald.” Additionally, three new species in the *Conchologia Indica* are named

“*theobaldi*,” which some modern authors would use to conclude that Theobald could not have been their co-author, given that current nomenclatural practice disfavors the naming of new species after oneself (Welters-Schultes, 2011). We refrain from attempting to extend modern ethical practices – which are non-binding even today – on nineteenth century zoology and have attributed all three “*theobaldi*” taxa to Hanley & Theobald. This work is also particularly problematic for having made available manuscript names of other authors, including Benson, disguising the fact that their first appearances were in this work.

It should be mentioned that another source of error in the earlier list of Hanley’s taxa by Norris & Dance (2002) was the incorrect dating of parts of the *Proceedings of the Zoological Society of London*, for which collations are available (Duncan, 1937; Cowan, 1973; Dickinson, 2005). We have indicated the correct date for each such part in the Literature Cited below.

Finally, one small undated pamphlet, *The shells of Margate, Ramsgate and Broadstairs*, was suggested by both Tomlin (1945) and Norris & Dance (2002) as possibly written by Hanley; Tomlin noted that Hanley possessed several copies in his library at his death. However, it was not authored by Hanley but

rather by Robert Chappell Osborne (a printer in Margate, who died in 1875), and is catalogued under Osborne's name in the Library of the Natural History Museum, United Kingdom (Osborne, 184_?). This tract cited Hanley (1840), so it must have appeared after 1840, and its figures were much cruder than those in Hanley (1840). Osborne also published other such tracts for Margate visitors (for example, Osborne, 1835). Perhaps Hanley provided Osborne with some advice, and was rewarded for his assistance with multiple copies of this sadly deficient effort.

History of Hanley's Collections

The identification of Hanley's type material has proven particularly problematic, given the dispersal of his collection, both before and after his death. Because Hanley was not employed at the British Museum or any other institution, he was under no obligation to deposit his type material in any one of them. Some of his new species were based on specimens already in the British Museum collection, and may be found there now. The majority of his new species, however, were based on material in his own collection, that of Hugh Cuming, and of several other contemporary shell collectors.

Hanley maintained a close relationship with Hugh Cuming and relied extensively on his collection in describing new species (concerning Cuming: Dance, 1980). Most of Cuming's collection came to the Natural History Museum, U.K., primarily through a purchase in 1866 at the price of £6,000, comprising 82,992 specimens (Smith, 1906: 710). Hanley also retained some specimens from the Cuming collection that later ended up in the Leeds City Museum. Hanley sometimes gave the localities attributed to Cuming's material in detail and sometimes not. In some cases, Cuming is inferred to be the collector of a species for which his name is not given because it is among others attributed to his collection and from a locality he is known to have visited. Coan (2011) discussed Cuming's eastern Pacific localities.

Another person who was a source of several species named by Hanley was William Metcalfe (18??–1874) (Tomlin, 1944), of whom nothing else is known, and only one (*Cytherea cor*) of the type specimens based on his material was located in 2012. Hanley also described material from the collections of Thomas Lombe Taylor (1802–1874), the Reverend Francis John Stainforth (1797–1866), and William Walton,

some specimens of which are extant in the Natural History Museum, U.K.

During Hanley's life, some of his type material entered the shell trade. One dealer, Robert F. Geale, whose shop was located at 55 Great Russell Street, just across from the British Museum, is known to have acquired specimens from Hanley. Geale started out as a secretary to Cuming, and later opened his own shop (Sheets-Pyenson, 1988: 129). In the early 1870s, Geale traded several Hanley type lots to John Gould Anthony (1804–1877), who was the Curator of Mollusks at the Museum of Comparative Zoology, Harvard University, and those types are now extant in that collection.

Hanley also traded specimens with Sven Lovén (1809–1895), curator of Mollusca at what is now the Swedish Museum of Natural History, Stockholm. Lovén was particularly interested in the cold-water marine fauna of northern Europe, and some of Hanley's type specimens from the region remain extant in that museum (A. Warén, pers. comm. 11 Jan. 2012).

It should be noted that the National Museum of Natural History, Smithsonian Institution (USNM) has several lots of Hanley species labeled as the "figd. type." With one exception (*Natica kingii*, now in the Lithoglyphidae), these are actually the specimens that were later figured by J. G. Jeffreys in his *British conchology* (1862–1869), and do not have any status as Hanley type material.

Hanley bequeathed his shell collection to his wife, who, in turn, "passed the collection into the care of a nephew" (Norris & Dance, 2002: 365). Nothing further is known of this nephew, Crew Hanley, other than that he had no interest in mollusks. According to Smith (1906: 723, 728) and Sherborn (1940: 65), Crew Hanley gave 104 type lots to the British Museum (Natural History) in 1900, but the rest were sold to William Harvey, a shell dealer whose shop was located on Houndsditch Road, due north of the Tower of London. Smith (1906: 728) recorded that Harvey "did not wish to part with any further portions of it." However, over the next decade, the British Museum (Natural History) was able to acquire several large selections of the Hanley collection from Harvey, which were accessioned in 1907 and 1912. The Hanley collection also included types of several species described by Reeve and by the Sowerbys. Most of the type specimens were incorporated into the type collection over the ensuing decades; one of us (Kabat), in June 2012, identified a number of additional type specimens in the

general collection that have now been transferred into the type collection.

It is not known what Harvey did with the remaining specimens after 1912, although by the early 1920s, they were placed in storage at T. R. Roberts, Ltd. (Norris, 1995: 20), a store in Islington (London) later acquired by the House of Fraser, a British department store chain. In 1932, what was left of the collection was presented to the Huddersfield Museum – a small municipal museum in a market town in southern Yorkshire (Norris & Dance, 2002: 365–366). Evidently, the Hanley collection made no impression on the staff at the Huddersfield Museum, for the history of that institution makes no mention of it (Davies, 1992). In 1957, the Huddersfield Museum transferred the Hanley collection to the Leeds City Museum, one of the two largest museums in Yorkshire (the other being the Yorkshire Museum in York, but that institution's molluscan collections are primarily fossils). Norris & Dance (2002: 366) discussed the move of the collection in 1957, the initial efforts by Dance, who was then employed at the British Museum (Natural History), to identify and transfer some of Hanley's type material to the British Museum (Natural History) over the next several years, and the attempts by Norris (who became curator of Natural History at Leeds) and others to curate the remaining parts of the Leeds collection commencing in the late 1970s. It should be noted that Dance, in 1960, initially concluded "that holotypes and figured specimens, even if they are not here [at the Natural History Museum], are unlikely to be at Leeds. From almost every tablet of types, the figured specimens [*sic*] has been removed" (I. C. J. Galbraith, Head of the Mollusca Section, memorandum to F. C. Fraser, Keeper of Zoology, "Sylvanus Hanley Collection – Leeds City Museum," 16 Feb. 1960). However, this conclusion was premature, as a number of holotypes and figured specimens did, in fact, remain in Leeds. Norris (1989: 52) correctly stated that the Hanley collection "is perhaps the most important of all the collections held by the [Leeds City] museum." Norris (1995: 20) explained that when the collection was transferred to Leeds in 1957, "Due to the lack of information ... and some bad advice and guidance in the late 1950s, a considerable amount of irreparable damage was done to the collection because many of the original labels were lost, and as a result, some of the original type and figured specimens are now untraceable."

At the Leeds City Museum, the Hanley collection was accessioned as No. 1957.173, and

catalog numbers were later assigned to 1,287 lots (1957.173.1 to 1957.173.1287), with some additional specimens catalogued in subsequent years. Yet others remain uncatalogued as of 2012. In the ensuing decades, parts of the Hanley collection were incorporated into the general collection, while the rest were kept together as part of a separate Hanley collection. In July 2012, one of us (Kabat) found and moved a number of the Hanley lots from the general collection back to the Hanley collection, thereby facilitating study of that material, and also identified a number of additional hitherto overlooked type specimens.

Hanley's library merits a brief mention. As an independent scholar, Hanley acquired a sizable working library, which enabled him to publish on a wide range of molluscan taxa. Hanley's library evidently remained largely intact after his death, for Ernest Ruthven Sykes (1867–1954) was able to purchase the book collection from a warehouse in London sometime before World War II (Dance, 1986: 189). In 1948, Sykes sold most of the library to Wheldon & Wesley, for prices that were a bargain even then, let alone today, as Dance noted with regret several decades later (Dance, 1986: 190–196; Swann, 1956: 122–123). Richard W. Foster and Richard I. Johnson, two malacologists associated with the Museum of Comparative Zoology, were able to buy many of the books in 1948 for their libraries, and for that of the Museum of Comparative Zoology at Harvard University (Johnson, 2006: 137). It is worth noting the fluctuation in prices for several titles. In 1890, one year after Hanley's death, the *Photographic conchology* could be had for 2 shillings 6 pence, and the *Conchologia Indica* for £3 17 shillings 6 pence (a reduction from the original price in 1876 of £8 5 shillings), while the Forbes & Hanley, *History of British Mollusca* with color plates was £11 11 shillings (Wesley, 1890: 8, 15, 19). In 1948, nearly six decades later, the same dealer had the *Photographic conchology* for £2 10 shillings, Hanley's personal copy of the *Conchologia Indica* for £12, and an uncolored set of the *History of British Mollusca* for £2 10 shillings (Dance, 1986: 192). (Note: prior to 1971, there were 20 shillings to the pound, and 12 pence to the shilling, i.e., 240 pence to the pound.) Today, the *Photographic conchology* is known from seven institutional libraries, while the *Conchologia Indica* is known from over 30 institutional libraries, and the *History of British Mollusca* from numerous libraries, according to the OCLC Worldcat database.

Type Material

The dispersal of Hanley's material, compounded with the poor curation of his own collection after his death, meant that it has not been easy for subsequent authors to determine with certainty whether a given specimen is, in fact, Hanley's original type material. The following factors contributed to this problem. First, Hanley seldom labeled his specimens as "types," and Harvey (or an unknown curator) replaced nearly all of Hanley's original labels with his own on the specimen boxes. Second, Hanley usually gave measurements for each species – and we found those measurements to be reasonably accurate when a species was based on a single specimen or when the measurement was of the figured specimen – but in other cases, those measurements appear to be "averages" of large and small specimens. Salisbury (1934: 91) noted this in remarking that "Hanley appears to have been very casual in the way of giving measurements, and frequently these seem to be a combination, or average, taken from the type lot." Third, while some of Hanley's figures are sufficiently detailed that they can be matched to a particular specimen (i.e., the figured syntype), others, particularly in the three editions of his monograph on the *Bivalvia*, were often reduced or enlarged in order to fit the same number of shells on each plate, and some may even be composites.

Since Hanley described many species from the Cuming collection, the curation of that collection deserves special mention, given that some of what has been published in the past is now known to be incorrect. Breure & Ablett (2011: 5–8, 11) provided a helpful discussion of how the Cumingian and other specimens were curated and re-curated over the decades: (1) Initially, the locality information was written on a loose slip of paper stuffed inside the shell; (2) later, these slips of paper were glued to wooden tablets, with the shells glued to the opposite side of the tablets; (3) some of the shells were then transferred from the wooden tablets to glass-topped cardboard boxes, with the locality information re-written on the bottom of the boxes and on a new label inside the box; (4) some of the cardboard boxes were cut off so that only the bottom portion remains, with the shell and cardboard label transferred to an open specimen box or an archival plastic bag; and, most recently (5) some of the labels were soaked off the cardboard, and transferred with the shell to an archival plastic bag. Examples

of all five curatorial methods can be found in the Natural History Museum today. Errors in taxonomic names or localities could have occurred while re-writing the labels.

Three provisions of the *International Code of Zoological Nomenclature* (ICZN, 1999) were applied in determining the type status of Hanley's type material. Of course, it must be recognized that the *Code* did not exist in Hanley's time, so Hanley can hardly be blamed for the fact that his descriptions require careful scrutiny under the current *Code* provisions. Further, the current provisions regarding lectotype designations did not exist until the 1985 edition of the *Code*, so that several authors prior to 1985 inadvertently designated lectotypes by referencing a specimen as the "holotype" when it was one of several syntypes.

ICZN *Code* Article 73.1, governing holotypes, makes clear that a holotype can only be inferred if the original description is clearly based on a single specimen, or the author used the term "holotype" or "the type." Hanley did not use either term in his descriptions, and he seldom indicated that a new species was based on a single specimen. When he did, that specimen, if it can be found, is the holotype. Since Hanley did not use the terms "holotype" or "the type," that means that there is no such thing as a Hanley "paratype," which is only used when the original description specified that there was a holotype or "the type" (Article 72.4.5).

If the original description was either expressly based on multiple specimens, or the original description gave no indication as to the number of specimens, then ICZN *Code* Article 73.2 requires that the type material, if found, be treated as syntypes. Thus, even if only one specimen is extant, it must be treated as a syntype. For many of Hanley's new species, he indicated that the species was known from more than one locality, or was present in more than one collection. Several other new species had descriptions that clearly referred to multiple specimens, sometimes by contrasting juveniles with adults, or indicating a range of color patterns in different specimens. However, the remaining new species had no indication as to the number of specimens, and while they could have been based on only one specimen, any such specimen must be treated as a syntype, not a holotype. Taken together, the majority of Hanley's new species fall under the *Code*'s Article 73.2, and their type material, if found, must be treated as syntype(s), even if only one specimen is now extant.

The ICZN *Code*'s provisions governing lectotypes have proven particularly problematic for researchers and curatorial staff. Prior to the 1980s, it was common for subsequent revisers to identify the figured specimen as the "holotype," and the remaining specimens as "paratypes." For Hanley's new species, such an act would be incorrect, since the only Hanley species for which there are holotypes are those that were clearly based on a single specimen. For those authors who published prior to 2000, ICZN *Code* Article 74.5 provides that the use by a subsequent author of the term "holotype" is *not* a valid lectotype selection "when the original work reveals that the taxon had been based on more than one specimen ... unless the author, when wrongly using that term, expressly indicated that he or she was selecting from the type series that particular specimen to serve as the name-bearing type." In contrast, ICZN *Code* Article 74.6 provides that if "it has been accepted that a nominal species-group taxon was based on a single specimen *and* the original description neither implies nor requires that there were syntypes, *and* if it is considered subsequently that the original description was based on more than one specimen" (emphasis added), then a pre-2000 citation of a specimen as the "holotype" is a valid lectotype designation.

Finally, for lectotype designations made after 1999, ICZN *Code* Article 74.7 requires even greater specificity in the designation, but that only affects a few of the Hanley species. It should be noted that ICZN *Code* Article 74.7.3 was amended in 2003 (retroactively to 1 January 2000) to require that such lectotype designations "contain an express statement of deliberate designation (merely citing a specimen as 'lectotype' is insufficient)." (ICZN Declaration 44, 2003). ICZN Recommendation 74G was also added in 2003, to make clear that for lectotypes, "The designation of lectotypes should be done as part of a revisionary or other taxonomic work to enhance the stability of nomenclature, and not for mere curatorial convenience." (Article 75.2 similarly prohibits neotype designations as a matter of convenience.)

As will be seen in the catalog below, the current provisions of the ICZN *Code* have rendered invalid the statements by a number of pre-1980 authors that a certain specimen was the "holotype" of a Hanley species, while treating such statements for yet other species as lectotype designations. For example, the

discussion of *Tellina* type material by Salisbury (1934) contains both inadvertent lectotype designations and erroneous identifications of holotypes. Salisbury (1934: pl. 11, fig. 5), identified a specimen as the "holotype" of *Tellina ancilla* Hanley, 1844. The lot contains two specimens, while the original description satisfies ICZN *Code* Article 74.6, so that Salisbury's action constitutes a valid, inadvertent lectotype designation. However, Salisbury (1934: pl. 13, figs. 7–9) identified another specimen as the "holotype" of *Tellina hiberna* Hanley, 1844 – a species that was clearly based on more than one specimen, from two localities, so that the original description instead satisfies ICZN *Code* Article 74.5, and Salisbury's action was not a valid lectotype designation. Numerous other examples of lectotype designations, valid or invalid, are set forth in the catalog below.

Just as it is no longer considered routine to designate a lectotype in the absence of a need to clarify the concept of a species, it is no longer routine to "restrict", "designate" or clarify a type locality when the original was broadly stated or unknown. (The term "clarify" is used in Recommendation 76A of the present *Code*.) In the cases of two Hanley taxa, subsequent authors in the 1950s and 1960s made such "restrictions", as was routine then. However, now it is often prudent to clarify a type locality when the original locality was unknown, and important to do so when it was incorrect. Such restrictions are often invalid because the type locality is tied to the primary type(s) under ICZN Article 76.1. These tasks are best done in the context of revisionary work, and we have not done so in this paper.

Hanley's Taxa

Hanley introduced 375 new species-group names for mollusks (255 in the Bivalvia and 120 in the Gastropoda), of which 367 are available and 8 are *nomina nuda*. Of the 367 available names, 219 (167 in the Bivalvia and 52 in the Gastropoda) are currently considered valid or are probably valid, or have not yet been assessed in the modern systematic literature (other than in uncritical checklists), and 148 (86 in the Bivalvia and 62 in the Gastropoda) are now known to be junior synonyms, junior homonyms, or have been treated as *nomen dubia*. An additional 53 species-group names were described by other authors, primarily Lovell Reeve, from Hanley manuscript names, or were erroneously attributed to Hanley by other authors.

Type material is known for 225 of the 367 available new species (176 in the Bivalvia, or 69% of his described bivalve species; 49 in the Gastropoda, or 41% of his described gastropod species), leaving 142 of the new species as yet without type material (77 in the Bivalvia and 65 in the Gastropoda). It should be noted that three of Hanley's bivalve species are represented by neotypes (one species each in the Nuculanidae, Ostreidae, and Solenidae), which still leaves 173 bivalve species (68%) represented by original type material.

Hanley also introduced two family-level names, both in the Gastropoda, of which one is in current use (Cancellariidae), the other now a junior synonym (Elysiidae). Hanley introduced six genera, of which two are in current use (*Cerithiopsis* and *Propilidium*, both in the Gastropoda) and four are junior synonyms (*Cavolina*, *Cochlycopa*, *Lucinopsis* and *Pilidium*); an additional eight generic names were errors by Hanley or were erroneously attributed by others to Hanley.

We have included the one taxon named by Charles Thorpe in the 1844 book that some authors attributed to Hanley. We have not included taxa described by other authors, such as Alder, expressly made available as by those authors within Forbes & Hanley (1848–1853), the taxa in Bean's Appendix to Thorpe (1844), nor any of the non-mollusks, such as tunicates, proposed by Forbes & Hanley.

Whereas most of Hanley's taxa were noted by him as being new, some were erroneously attributed by Hanley, or by Hanley & Theobald, to other workers but were in fact first made available by Hanley. It is possible that there remain yet other unrecognized Hanley taxa, such as in his lengthy edition of Wood's *Index testaceologicus* (Hanley, 1856b), but none have come to our attention as being from that work.

The entries are grouped by molluscan class and family, in systematic order following Bouchet & Rocroi (2005, 2010), and are alphabetical within each family, with the true Hanley names listed first. The Hanley work or works covering the names are in chronological order, keyed to the Literature Cited entries. If a taxon first became available in a work other than the first one noted, such as in a caption or description published later than an uncaptioned illustration, this entry is underlined. The type locality is given, with bracketed notes added for some to clarify Hanley's terminology or to provide updated information on modern geo-

graphical names. Information on the modern interpretation of the taxon is given, as we noted in the literature available to us. Finally, if type material is known, we specify its museum(s) and catalogue number(s).

The following is a list of the taxa that were introduced by Hanley, both available and *nomina nuda*, whether or not currently considered valid, as well as names that have been incorrectly attributed to him. His names are in boldface at the beginning of each family. Those now considered valid, or that are probably or possibly valid, are indicated by an asterisk. Those lacking an asterisk are considered synonyms of earlier taxa, or are junior homonyms that have been superseded by later synonyms or replacement names.

The list of taxa not in boldface under each family and with "Hanley" in quotation marks, are those misattributed to Hanley by Sherborn, Reeve or other authors. We have not included names expressly indicated by Sherborn as merely being transfers by Hanley from other genera of earlier, non-Hanley species. The list also contains some Hanley misspellings or invalid emendations of the names of earlier taxa. It should be noted that when Hanley described a taxon as a "variety" ("var.") of a species, he followed the classical tradition in giving the varietal name the gender of "varietas" and not of the genus in which the variety was described, which explains why some of Hanley's varieties do not match the gender of the genus.

Acronyms

Leeds Museum	Leeds City Museum, Leeds, U.K.
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, U.S.A.
NHM	UK Natural History Museum of the United Kingdom, London [formerly British Museum (Natural History)], U.K.
SMNH	Swedish Museum of Natural History, Stockholm, Sweden
USNM	National Museum of Natural History, Washington, D.C., U.S.A.
*	Names considered valid (i.e., neither a junior synonym nor a junior homonym that have been replaced).

BIVALVIA
Nuculidae

Nuculanidae

antipodum, *Nucula* – Hanley, 1860c: 159–160, 166, pl. 230, fig. 155; 1861b: 441. Australia; Hanley coll. Synonym of *Leionucula obliqua* (Lamarck, 1819) (Lamprell & Healy, 1998: 34–35, fig. 12). The generic name *Ennucula* is now preferred over *Leionucula* (Huber, 2010: 523). Type material not found in NHMUK or Leeds Museum in 2012.

malabarica, *Nucula – Hanley, 1860c: 155–156, 167, pl. 230, fig. 152; 1861b: 441. Cochin, Malabar, India; Hanley coll. Listed by Prashad (1933: 129) as a valid species, but one that he had not seen. Type material not found in NHMUK or Leeds Museum in 2012.

mandraei, *Nucula* – Hanley, 1860c: 161–162, 167, pl. 229, fig. 143, as “*m’andraei*”; 1861b: 441–442, as “*m’andrewii*”. Tunisia; M’Andrew (Hanley, 1861b); Cuming coll. There was nothing in the original account to indicate that the species was intended to honor M’Andrew, and thus the original misspelling must stand (ICZN Code Art. 32.5). Synonym of *Ennucula aegeensis* (Forbes, 1844) (Sabelli et al., 1990: 274, as “*macandrewii*”). NHMUK 1992167, four possible syntypes.

mauritii, *Nucula* – Hanley, 1859c: 237, *nomen nudum*. Mentioned as “(Hanley as of Hinds), Recent Bivalves”, but not located therein.

radiata, *Nucula* – Hanley, in Forbes & Hanley, 1849: 220–221, pl. 47, figs. 4, 5, pl. 48, fig. 7; Hanley, 1860c: 149–150, 168, pl. 229, figs. 123, 124. *Non Nucula radiata* De Kay, 1843, *non N. radiata* Portlock, 1843. Renamed *Nucula hanleyi* Winckworth, 1931. South Devon, Torbay (Alder & Hanley). Milford Haven, in 10 fms. [18 m], “always in separate parts of the bay from *nucleus*” (M’Andrew & Forbes); Lundy Island, 7 to 25 fms. [13–46 m], in sandy-gravel (M’Andrew). Lamlash, Arran (Alder); “and, probably, Exmouth (Clark)”. *Nucula (Nucula) hanleyi* Winckworth, 1931 (Tebble, 1976: 27; Huber, 2010: 86). NHMUK 1848.10.13.22–25, four possible syntypes (M’Andrew collection); NHMUK 1992166, four possible syntypes (South Devon, Teignmouth); SMNH 3657, syntypes. USNM 1917180, from the Jeffreys collection, is labeled as the “figured type,” but this is merely the specimen figured by Jeffreys in his *British conchology* (1869: pl. 29, fig. 2a).

brookei, *Leda – Hanley, 1860c: 132–133, 166, pl. 229, fig. 106; 1861b: 440, as *L. brookii*. Borneo; Cuming coll. Type material not found in NHMUK or Leeds Museum in 2012.

confusa, *Leda – Hanley, 1860c: 119–120, 166, pl. 228, fig. 85. Japan, China. *Saccella confusa* (Hanley, 1860) (Xu, 2004: 211; Huber, 2010: 96). NHMUK 1996503, three possible syntypes (largest was figured by Hanley, 9.2 mm x 16.5 mm; also figured by Higo et al., 2001: 147, fig. B69).

conradi, *Leda – Hanley, 1860c: 130–131, 166, pl. 229, fig. 101; 1861b: 440. Loc. unknown; Taylor coll. NHMUK 1879.2.26.239, holotype (4.4 mm x 8.8 mm).

dohrni, *Leda – Hanley, 1861c: 242. Pacific Ocean; Dohrn. The eastern Australian *Nuculana (Scaeoleda) dohrni* (Hanley, 1861) (Lamprell & Healy, 1998: 40, fig. 38). Type material not found in NHMUK or Leeds Museum in 2012.

gibbosa, *Leda elenensis* var. – Hanley, 1860c: 121–122. *Non Nucula gibbosa* G. B. Sowerby I, 1833, then regarded as belong in the same genus. Hanley’s variety is now regarded as a synonym of *Saccella elenensis* (G. B. Sowerby I, 1833) (Coan & Valentich-Scott, 2012: 76). It is difficult to explain why, in discussing his new variety, Hanley cited Sowerby’s original figure of *Nucula gibbosa*, which Hanley had listed as a separate species of *Leda* on the previous page. Perhaps it was to indicate its analogous width, hence the varietal name (see also under *media* below). (Sowerby’s *Nucula gibbosa* is a primary junior homonym of *Nucula gibbosa* Fleming, 1828, with the name in current use for Sowerby’s taxon being *Saccella fastigata* (Keen, 1958) – Coan & Valentich-Scott, 2012: 76.) Type material not found in NHMUK or Leeds Museum in 2012.

hindsii, *Leda – Hanley, 1860c: 135, 167, pl. 229, fig. 102; 1861b: 440–441. Golfo de Nicoya, Costa Rica; probably collected by H. Cuming; Cuming, Hanley & Metcalfe colls. The tropical eastern Pacific *Saccella hindsii* (Hanley, 1860) (Coan & Valentich-Scott, 2012: 76–77, as “1861”). SBMNH 359319, neotype from Puntarenas on the Golfo de Nicoya (Coan & Valentich-Scott, 2012).

media, *Leda elenensis* var. – Hanley, 1860c: 121–122. On p. 122, Hanley cited a figure in his catalogue, “(*Nuc. cuneata*, Hanley,

Rec. Bivalves, pl. 19, f. 57, not description)"; however, he evidently meant to cite fig. 58, i.e., his figure of *N. cuneata* G. B. Sowerby I, 1833, from Chile. Here again the citation may have been intended to indicate an analogous shape. The variety *media* is regarded as a synonym of *Saccella elenensis* (G. B. Sowerby I, 1833) (Coan & Valentich-Scott, 2012: 76). Type material not found in NHMUK or Leeds Museum in 2012.

metcalfei, *Leda – Hanley, 1860c: 110–111, 167, pl. 227, fig. 34; 1861a: 370, as *L. metcalfii*. Philippine Islands; Cuming & Hanley colls. This is an *Adrana*, a genus found only the New World, so the type locality was probably incorrect. It is possibly an older name for *Adrana exoptata* (Pilsbry & Lowe, 1932), from the tropical eastern Pacific (M. Huber, 2010: 532). NHMUK 20030195, syntype (too fragile to measure) (original description refers to "only two" specimens; second syntype not found).

micans, *Leda – H. Adams & A. Adams, 1858: 547, *nomen nudum*; Hanley, 1860c: 130, 167, pl. 229, fig. 100, ex A. Adams ms. "New Zealand"; [Cuming coll.]. Not mentioned in Powell (1979). Beu (2006: 172) examined the "holotype" (labeled as having come from the Cuming collection), and suggested that this taxon is an earlier name for the northeast Australian *Saccella darwinii* (E. A. Smith, 1884). Huber (2010: 531) further suggested that both merit close comparison with *S. inconspicua* (A. Adams, 1856), also from Australia, which may be a still earlier name. NHMUK 20040714, syntype (Hanley's description does not indicate whether it was based on one or more specimens).

producta, *Leda confusa var. – Hanley, 1860c: 120. Type locality not specified; presumably that for *Leda confusa* Hanley, 1860, described from "Japan, China." Type material not found in NHMUK or Leeds Museum in 2012.

pyriformis, *Leda elenensis* var. "?" – Hanley, 1860c: 121–122. Synonym of *Saccella elenensis* (G. B. Sowerby I, 1833) (Coan & Valentich-Scott, 2012: 76). Type material not found in NHMUK or Leeds Museum in 2012.

taylori, *Leda* – Hanley, 1860c: 109–110, 168, pl. 227, fig. 35; 1861a: 370. Guatemala; Cuming & Taylor colls. Synonym of the tropical eastern Pacific *Adrana crenifera* (G. B. Sowerby I, 1833) (Coan & Valentich-Scott, 2012: 61, as "1861"). NHMUK 20030194, two syntypes (Cuming collection).

Malletiidae

brevior, *Solenella norrisii* var. – Hanley, 1860c: 164. Valparaiso, Chile; Cuming coll. Presumably a synonym of *Malletia chilensis* Des Moulins, 1832, of which *Solenella norrisii* G. B. Sowerby I, 1833, is a synonym. Type material not found in NHMUK or Leeds Museum in 2012.

cumingii, *Solenella – Hanley, 1860c: 164–165, 166, pl. 226, fig. 3; 1861b: 441. Falkland Islands; Cuming coll. *Malletia cumingii* (Hanley, 1860), which occurs in the Falkland Islands and adjacent southeastern South America (Huber, 2010: 539; D. Zelaya, pers. comm., 31 May 2012). Type material not found in NHMUK or Leeds Museum in 2012.

Yoldiidae

woodwardi, *Leda (Yoldia) – Hanley, 1860c: 140–141, 168, pl. 226, figs. 17, 22; 1861a: 370–371, as *Yoldia*. Falkland Islands; Cuming, Hanley & Taylor colls. Dell (1964: 146–147, pl. 2, fig. 11) treated this as a junior synonym of the circum-Antarctic *Yoldia (Aequiyoldia) eightsi* (Couthouy, in Jay, 1839). However, Rabarts & Whybrow (1979) concluded that Hanley's name was a distinct species of *Yoldia*. NHMUK 19764 and NHMUK 19765, two syntypes (Falkland Islands, Cuming collection) (figured by Rabarts & Whybrow, 1979: 180, fig. 16).

Mytilidae

arcuatula, *Modiola – Hanley, 1843g: 236; 1844b: 16. Philippine Islands (1843g); Singapore; low water (1844b). *Arcuatula arcuatula* (Hanley, 1843) from Southeast Asia (Lyngé, 1909: 133; Huber, 2010: 112). Type species of *Arcuatula* Jousseaume in Lamy, 1919, by tautonymy. Soot-Ryen (1955: 55) erroneously stated that the type species of this genus was *Modiola plicata* Lamarck, 1819. This is not the brackish and freshwater species discussed and figured by Brandt (1974: 257–258, 380, pl. 18, fig. 21) as "*Brachidontes arcuatulus* (Hanley)" from Thailand, which is instead *Brachidontes evansi* (E. A. Smith, 1903). Type material not found in NHMUK or Leeds Museum in 2012.

biradiata, *Modiola – Hanley, 1843g: 235–236; 1844b: 15. "South America?". *Gibbomodiola biradiata* (Hanley, 1843) from the western Indo-Pacific (Huber, 2010: 124). Type material not found in NHMUK or Leeds Museum in 2012.

canaliferus, *Modiola (Lithodomus) – Hanley, 1843g: 239; 1844b: 16–17, as *Lithodomus*; 1856a: 387, 23 (pl. expl.), pl. 24, fig. 22. Zebu, Philippine Islands; on rocks. *Lithophaga (Diberus) canalifera* (Hanley, 1843) (Kleemann, 1983: 4–5). NHMUK 1907.10.28.52, lectotype (selected by Kleemann, 1983: 4–5); NHMUK 1907.10.28.53, paralectotype; Leeds Museum 1957.173.24634, three paralectotypes.

cuneiformis, Modiola – Hanley, 1843g: 237; Philippine Islands, as “Zool. P. 1844”, but not published therein. Later, Hanley (1856a: 387–388) noted that he had mistakenly inserted under this species name on p. 237 a description of *Modiola elongata* (Swainson, 1821). That being the case, and without an original figure, this would make *M. cuneiformis* either (1) a synonym of *M. elongata*, or (2) a name for what Hanley had in hand that he thought was a new species, in that Hanley’s later figure (1856a: pl. 24, fig. 28) has been interpreted as a specimen of the Indo-Pacific *Jolya rhomboidea* (Reeve, 1857) (Huber, 2010: 556–557), assuming that Reeve’s species can be distinguished from the former based on Hanley’s description alone. (See also under *elongata, Modiola* below.) Type material not found in NHMUK or Leeds Museum in 2012.

granulatus, *Mytilus – Hanley, 1843g: 246; 1844b: 17; 1856a: 388, 24 (pl. expl.), pl. 24, fig. 33. “S. America” (1843g); Valparaíso, Chile; under rocks at low water; H. Cuming (1844b). [Non *Modiola granulata* Potiez & Michaud, 1844: 132, pl. 54, fig. 10, a fossil from an unknown locality]. The 1843g entry was listed in error by Sherborn (1926: 2808) as having been proposed in *Modiola. Brachidontes granulatus* (Hanley, 1843) from Peru and Chile (Forcelli, 2000: 149, as “1844”; Huber, 2010: 117). NHMUK 1900.2.8.13–18, six syntypes (“Chili”).

metcalfei, *Modiola – Hanley, 1843g: 235; 1844b: 14; 1856a: 387, 23 (pl. expl.), pl. 24, fig. 25. Philippine Islands. The western Pacific *Modiolus metcalfei* (Hanley, 1843) (Lynge, 1909: 131; Hylleberg & Kilburn, 2002: 58; Wang, 2004a: 230, pl. 122, fig. G); however, older names for this species may be *Modiola microptera* Deshayes, 1836, and *Musculus modulaides* Röding, 1798 (Huber, 2010: 554). NHMUK 196589 and NHMUK 196590, two syntypes (Higo et al., 2001: 148, fig. B110, as “lectotype,” in error). NHMUK 196588 was determined by B.R. Wilson (1982, note

in collection) not to be a type specimen, and NHMUK 1907.10.28.108 was determined by S. Morris (1982, note in collection) to be a different species.

philippinarum, *Modiola – Hanley, 1843g: 235; 1844b: 15; 1856a: 387, 23 (pl. expl.), pl. 24, fig. 26. Zebu, Philippine Islands. The western Pacific *Modiolus philippinarum* (Hanley, 1843) (Lynge, 1909: 132; Hylleberg & Kilburn, 2002: 58; Wang, 2004a: 230, pl. 122, fig. I; Huber, 2010: 123). NHMUK 19829, three syntypes (NHMUK 19829/1 was figured by Reeve, 1858, pl. 1, fig. 1, and by Higo et al., 2001: 148, fig. B111); Leeds Museum 1957.173.24629, syntype (Hanley’s figured specimen, 91.7 mm x 45.6 mm); Leeds Museum 1957.173.24630–24632, three possible syntypes.

plumula, *Modiola (Lithodomus) – Hanley, 1843g: 239–240; 1844b: 17, as *Lithodomus*; 1856a: 387, 23 (pl. expl.), pl. 24, fig. 23. Philippines (1843g); Panama; in *Spondylus* (1844b), the latter probably correct. The tropical eastern Pacific and Indo-Pacific *Lithophaga (Diberus) plumula* (Hanley, 1843) (Coan & Valentich-Scott, 2012: 137–138). *Modiola plumula* Hanley, 1843, is the type species of *Diberus* Dall, 1898, by original designation. NHMUK 1907.10.28.50, lectotype (selected by Kleeman, 1983: 20); NHMUK 197595, three paralectotypes; Leeds Museum 1957.173.24633, one paralectotype.

sordida, Modiola – Hanley, 1843g: 236; 1844b: 16. Loc. unknown; Metcalfe coll. A type specimen has not been located, and there was no figure, so this is best regarded as a *nomen dubium* (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

striatula, *Modiola – Hanley, 1843g: 241; 1844b: 14; 1856a: 388, 23 (pl. expl.), pl. 24, fig. 29. Batangas, Philippine Islands. *Brachidontes striatulus* (Hanley, 1843) from Southeast Asia (Huber, 2010: 118). NHMUK 1907.10.28.54, figured syntype (Higo et al., 2001: 148, fig. B97); NHMUK 1965106, three syntypes; NHMUK 1985010, three syntypes. Morton & Tan (2003: 143) stated that the “holotype” is in the Leeds Museum, but no such specimen was found in 2012.

strigata, *Modiola – Hanley, 1843g: 243; 1844b: 15–16. Sibango, Zebu; 10 fms. [18m], mud. The southeast Asian *Musculus strigatus* (Hanley, 1843) (Huber, 2010: 111). Type material not found in NHMUK or Leeds Museum in 2012.

strigatus, *Mytilus* – Hanley, 1843g: 251, ex Hinds ms, as “Z. P. 1844”, but not published in therein; 1856a: 388, 24 (pl. expl.), pl. 24, fig. 34. Loc. not stated. Synonym of *Mytella charuana* (d’Orbigny, 1842) (Coan & Valentich-Scott, 2012: 115). NHMUK 1907.12.30.339, holotype (40.8 mm x 16.2 mm).

subramosa, *Modiola – Hanley, 1843g: 241; 1844b: 14–15; 1856a: 388, 23 (pl. expl.), pl. 24, fig. 30. Cagayan, Mindanao, Philippine Islands. The Indo-Pacific *Brachidontes subramosus* (Hanley, 1843) (Lyngø, 1909: 134, as “1844”; Huber, 2010: 118). NHMUK 1907.10.28.49, figured syntype (31.4 mm x 12.6 mm); NHMUK 1963440, three syntypes.

tumida, *Modiola* – Hanley, 1843g: 241. England. This unfigured species, not indicated as being new, was based on eight earlier references to *Mytilus discors* Linnaeus, 1767, and two references to *Modiola discrepans* Lamarck, 1819 (*non M. discrepans* Montagu, 1803), together with a short description. Hanley thus distinguished the true *Modiolus discors* (Linnaeus) from the “discors” of the cited authors. Formerly treated as the eastern Atlantic *Modiolarca tumida* (Hanley, 1843) (Bowden & Heppell, 1966: 103, 116; S. M. Smith & Heppell, 1991: 60), but more recently regarded as a junior synonym of *Modiolarca subpictus* (Cantraine, 1835), which itself had earlier been regarded as a *nomen dubium* (Huber, 2010: 111). This symbiotic species resides within the tests of sea squirts (ascidians) (Roberts & Breen, 1985; Morton & Dinesen, 2011). Type material not found in NHMUK or Leeds Museum in 2012; because it was described from earlier references, the type material is that of those previous authors and any specimens available to Hanley.

brasiliensis, *Modiola* – “Hanley”, 1843g: 234. Guyana. This was intended to represent *Modiola brasiliensis* Gray, 1825. Gray (1825: 139) cited two figures in Chemnitz (1795: pl. 205, figs. 2018, 2019) of a species of *Pteria* instead of the adjacent figures he had intended to cite (figs. 2020, 2021). However, his additional citation of *Mytilus latus* Dillwyn (1817: 311–312), who under one of his synonyms cited the correct Chemnitz figures, as well as Gray’s placement of this species in *Modiola*, makes Gray’s intent clear. Gray’s species has been regarded as a synonym of *Mytella guyanensis* (Lamarck, 1819) (Coan &

Valentich-Scott, 2012: 115–117, who mistakenly credited *brasiliensis* to Hanley). However, Gray’s species may instead be a synonym of *Mytella bicolor* (Bruguère, 1792), which has recently been regarded as distinct, occurring both in the western Atlantic and eastern Pacific, with *Mytella guyanensis* (Lamarck, 1819) present only in the western Atlantic (Huber, 2010: 113, 547).

elongata, *Modiola* – “Hanley”, 1856a: 387–388, 23 (pl. expl.), pl. 24, fig. 28. Hanley (1856a) was well aware of the use of this name by Swainson (1821), having published the 1841 edition of Swainson’s *Exotic Conchology* himself. In 1856, Hanley noted that he had mistakenly inserted the description of *Modiola elongata* under his own *M. cuneiformis* (Hanley, 1843g: 23) (see above). *Modiola elongata* can thus not be taken as the proposal of another, homonymous species. Huber (2010: 556–557) considered that what Hanley illustrated as *M. elongata* Swainson was actually a specimen of the Indo-Pacific *Jolya rhomboidea* (Reeve, 1857).

nigra, *Modiola* – “Thorpe”, 1844: 249. Listed by Sherborn (1928: 4316) as also being by Thorpe, but is the same as *Modiola nigra* Gray, 1824, also listed by Sherborn.

patagonica, *Modiola* – “Hanley”, 1843g: 236, ex d’Orbigny ms. Patagonia. Hanley treated this species as if it were an unpublished manuscript name, but it had become available with publication of d’Orbigny’s plate 85 with caption in 1842; d’Orbigny’s corresponding text (pp. 646–647) did not appear until 1846. *Mytilus patagonicus* d’Orbigny, 1842, is probably a synonym of *Mytilus galloprovincialis* Lamarck, 1819.

rhomboidea, *Modiola* – “Hanley”. Reeve (1857: pl. 6, fig. 28) attributed this species to Hanley (“Species Recent Shells”), but Hanley did not name this Indo-Pacific species and it should be attributed to Reeve, who incorrectly indicated a West African origin.

Arcidae

deshayesii, *Arca* – Hanley, 1843f: 157 (footnote to description of *Arca antiquata*); 1843g: 371; 1844g: 16 (pl. expl.), pl. 18, f. 42. Also based on Gualtieri (1742: pl. 87B) and Bruguère (1798: pl. 306, fig. 1). Synonym of the western Atlantic *Anadara notabilis* (Röding, 1798) (Weisbord, 1964: 69–72, pl. 5, figs. 7–10). NHMUK 1907.10.28.126, “possible holotype” (Stevenson, 1973: 197) (44.0 mm x 28.3 mm).

Pteriidae

mazatlanica, *Meleagrina – Hanley, 1856a: 388, 24 (pl. expl.), pl. 24, fig. 40. Mazatlán [Sinaloa], Mexico. The tropical eastern Pacific *Pinctada mazatlanica* (Hanley, 1856) (Coan & Valentich-Scott, 2012: 216–217). Type material not found in NHMUK or Leeds Museum in 2012.

Isognomonidae

listeri*, *Perna – Hanley, 1843g: 259. West Indies. Also cited Lister (1687: pl. 228, fig. 63) and Klein (1753: pl. 8, fig. 19). Synonym of the western Atlantic *Isognomon radiatus* (Anton, 1838) (Lamy, 1933: 397; Tunnell et al., 2010: 320). NHMUK 1907.10.28.7, possible syntype (45.3 mm x 26.8 mm).

Pinnidae

carolinensis*, *Pinna – Hanley, 1858d: 225–226. Carolina, U.S.A. [“restricted” to Beaufort, North Carolina by Turner & Rosewater, 1958]; Cumming & Hanley colls. Synonym of *Atrina rigida* [Lightfoot, 1786] (Turner & Rosewater, 1958: 312–314, as “Solander”). Type material not found in NHMUK or Leeds Museum in 2012.

chemnitzii*, *Pinna – Hanley, 1858b: 136. Philippine Islands; H. Cumming; Cumming & Hanley colls. Rosewater (1961: 211–215) considered this to be a synonym of *Atrina pectinata* (Linnaeus, 1758). However, recent work suggests that it is instead a synonym of the Asian *Atrina chinensis* (Deshayes, 1841) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1952.8.29.12, syntype (Wilkins, 1953a: 27, erroneously stated that this was the “holotype,” but Hanley’s original description reveals that the taxon was based on more than one specimen, from two collections (ICZN Code Article 74.5) (measured by Wilkins as 280 mm x 128 mm); NHMUK 1952.8.29.13, syntype.

****hystrix*, *Pinna*** – Hanley, 1858d: 226. Amboyna; Cumming & Hanley colls. Mistakenly regarded as a synonym of *Atrina vexillum* (Born, 1778) by Rosewater (1961: 204–207), it is instead the distinct Indian Ocean *Atrina hystrix* (Hanley, 1858) (Lynge, 1909: 148–149; Huber, 2010: 165). NHMUK 1952.8.29.28–30, syntypes (three specimens and one loose valve; two are 2 inches longer than the measured length given by Hanley, and the others are much smaller). Wilkins (1953a: 27)

stated that the NHMUK lot consisted of “three paratypes,” and also referred to the “Holotype in Mus. Hanley (not located).” This was not a valid lectotype designation, because Hanley’s original description reveals that the taxon was based on more than one specimen (ICZN Code Article 74.5).

kraussii*, *Pinna – Hanley, 1858d: 226. Natal; Hamilton, Hanley & Sowerby colls. Synonym of *Atrina squamifera* (G. B. Sowerby I, 1835) (Rosewater, 1961: 207–210). Type material not found in NHMUK or Leeds Museum in 2012.

minax*, *Pinna – Hanley, 1858d: 227. “New Guinea? Mexico?”; Hanley coll. “Perhaps a strongly spined form” of *Atrina vexillum* (Born, 1778) (Winckworth, 1929: 293). No type material has been located, and there was no original or subsequent figure by Hanley, so this is best regarded as a *nomen dubium* (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

rostellum*, *Pinna – Hanley, 1858d: 227–228. Indian [Ocean] islands; Cumming & Hanley colls. Regarded by Rosewater (1961: 193–199) as a synonym of the rare Red Sea *Pinna bicolor* Gmelin, 1791. However, there was no original figure and no type material has been located, so it should be regarded as a *nomen dubium* (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

In his monograph on *Pinna*, Reeve (1858–1859) obtained a number of Hanley’s manuscript names, perhaps from labels in the Cumming collection, and published them before Hanley’s paper appeared. He also credited two taxa to Hanley that were not among those Hanley ever published. All these should be credited to Reeve, ex Hanley ms, not to Hanley in Reeve. Reeve’s Latin descriptions are all much shorter and very different, his English text is not that present in Hanley’s publication, and figures have been added. While it is clear that Reeve knew that a Hanley publication was in press, he may not have even seen a Hanley manuscript or proofs. The type material for these species in the NHMUK is all correctly labeled with Reeve as the author, and Wilkins (1953a: 27–29), in his type catalog, attributed them to Reeve alone. The species involved, all published in 1858, are *Pinna assimilis* (pl. 31,

fig. 59 – Reeve, Aug.; Hanley, Nov.), *P. cumingii* (pl. 16, fig. 29 – Reeve, May; Hanley, Nov.), *P. dorbignyi* (pl. 26, fig. 49 – Reeve, Aug.; Hanley, Nov.), *P. euglypta* (pl. 20, fig. 37 – Reeve, June; Hanley, Nov.), *P. fumata* (pl. 15, fig. 27 – Reeve, May; Hanley, Nov.), *P. gouldii* (pl. 11, fig. 21 – Reeve, May; Hanley, Nov.), *P. japonica* (pl. 25, fig. 47; never published by Hanley), *P. menkei* (pl. 18, fig. 34 – Reeve, June; Hanley, Nov.), *P. philippiensis* (pl. 11, fig. 20; never published by Hanley), *P. regia* (pl. 30, fig. 56 – Reeve, Aug.; Hanley, Nov.), *P. rumphii* (pl. 5, fig. 9 – Reeve, April; Hanley, July), and *P. strangei* (pl. 27, fig. 52 – Reeve, Aug.; Hanley, Nov.).

Ostreidae

bicolor, *Ostrea* – Hanley, 1846b: 107; 1854: pl. 1, fig. 2; 1856a: 305. “Senegal?”; Cuming & Hanley colls. Cited as a synonym of the west African *Crassostrea parasitica* (Gmelin, 1791) by Inaba et al. (2004: 20–21). Huber (2010: 180, 609) regarded Gmelin’s species to be a *nomen dubium* and Hanley’s taxon to be a synonym of *Crassostrea tulipa* (Lamarck, 1819). NHMUK 1912.6.4.2, figured syntype (72.7 mm x 59.9 mm) (Hanley’s description referred to “the few specimens,” so this is not a holotype).

callichroa, *Ostrea* – Hanley, 1846b: 107; 1856a: 305–306. Isla Chiloe, Chile; low-water, on rocks; H. Cuming; Cuming coll. Synonym of the Chilean *Tiostrea chilensis* (Philippi, 1844) (Inaba et al., 2004: 45–46, as Philippi, “1846”). More recently, Beu (2006: 210) and Huber (2010: 607) regarded *Tiostrea* as a synonym of *Ostrea*. Type material not found in NHMUK or Leeds Museum in 2012.

chemnitzii, *Ostrea* – Hanley, 1846b: 106; 1856a: 304–305. “China?”; Cuming & Hanley colls. Also cited Chemnitz (1786: pl. 116, fig. 994). Synonym of the Indo-Pacific *Dendrostrea rosacea* (Deshayes, 1836) (Inaba et al., 2004: 57–58). NHMUK 1912.6.4.1, syntype (figured by G. B. Sowerby II, 1871: 18, pl. 20, fig. 47b); NHMUK 1907.12.30.5, syntype (figured by G. B. Sowerby II, 1871: 18, pl. 20, fig. 47a); Leeds Museum 1957.173.24635, syntype (67.4 mm x 47.0 mm). Higo et al. (2001: 155, fig. B395s) illustrated the NHMUK type material and noted that these specimens may represent two species.

columbiensis, *Ostrea – Hanley, 1846b: 107; 1856a: 305. Santa Elena, West Colombia [now Guayas, Ecuador], half-tide, on rocks; H. Cuming; Cuming coll. The tropical eastern

Pacific *Crassostrea columbiensis* (Hanley, 1846) (Coan & Valentich-Scott, 2012: 239–240). SBMNH 120192, neotype (Coan & Valentich-Scott, 2012).

iridescens, *Ostrea* – Hanley, 1854: pl. 2, figs. 6, 7, ex Gray ms. Synonym of the eastern Pacific *Striostrea prismatica* (Gray, 1825) (Coan & Valentich-Scott, 2012: 243). NHMUK 1912.6.18.40, syntype.

lacerata, *Ostrea* – Hanley, 1846b: 106; Hanley, 1856a: 305, as “*O. lacerans*”. “Senegal?”; Cuming & Petit [de la Saussaye] colls. *Non* *O. lacerata* Goldfuss, 1833. The west African *Crassostrea lacerata* (Hanley, 1846) according to Inaba et al. (2004: 20–21), but it would have to be renamed as it is a junior homonym. However, Huber (2010: 613–614) considered it to be a synonym of the west African *Dendostrea senegalensis* (Gmelin, 1791). NHMUK 20080620, syntype (Senegal, Cuming collection) (49.0 mm x 40.1 mm) (as Hanley referred to multiple “specimens,” this is not a holotype).

megodon, *Ostrea – Hanley, 1846b: 106; Hanley, 1856a: 309. Peru; H. Cuming; Cuming coll. The tropical eastern Pacific *Ostrea megodon* Hanley, 1846 (Inaba et al., 2004: 41–42) or now preferably *Undulostrea megodon* (Hanley, 1846) (Coan & Valentich-Scott, 2012: 237–238). *Ostrea megodon* Hanley, 1846, is the type species of *Undulostrea* Harry, 1985, by original designation. NHMUK 20120168, four syntypes (four specimens comprising seven valves).

multistriata, *Ostrea* – Hanley, 1846b: 106; 1854: *Ostrea* pl. 1, fig. 3; 1856a: 305. Coast of Africa; Cuming, Hanley & Saul colls. *Non* *O. multistriata* Poli, 1795, *non* *O. multistriata* Deshayes, 1832. Renamed *Ostrea procellosa* Lamy, 1929 (pp. 71–72), ex Valenciennes ms. Synonym of the west African *Striostrea margaritacea* (Lamarck, 1819) (Inaba et al., 2004: 32, who placed this species in *Saccostrea*). *Ostrea procellosa* is the type species of *Striostrea* Vialov, 1936, by original designation (Stenzel, 1971: 1135–1138; Huber, 2010: 181). NHMUK 1907.12.30.335, figured syntype (60.6 mm x 55.1 mm); Leeds Museum 1957.173.24636, syntype (74.8 mm x 73.2 mm).

pestigris, *Ostrea – Hanley, 1846b: 106–107; 1856a: 309–310, both as “*pes-tigris*”; Luzon, Philippine Islands; H. Cuming; Cuming & Walton colls. The southwestern Pacific *Planostrea pestigris* (Hanley, 1846) (Hylleberg & Kilburn, 2003: 163; Inaba et al., 2004: 43–44). *Ostrea pestigris* Hanley, 1846, is the type species

of *Planostrea* Harry, 1985, by original designation. NHMUK 1907.12.30.333, possible syntype (figured in G. B. Sowerby II, 1871: pl. 30, fig. 78a, 78b; Higo et al., 2001: 155, fig. B406). Lam & Morton (2004: 21; 2009: 487) incorrectly stated that USNM 666809 was the “holotype” of this species; however, USNM 666809 is merely a specimen from Borneo that was illustrated by Harry (1985: 143, fig. 22).

“*purpurea?*”, *Ostrea edulis* var. – Hanley, 1854: *Ostrea*, pl. 2, fig. 8; 1856a: 296. Toulon, France; presumably Hanley coll. Homonym of *Ostrea purpurea* Lightfoot, 1786. Synonym of *Ostrea edulis* Linnaeus, 1758 (Inaba et al., 2004: 33–36). NHMUK 1912.6.18.42, figured holotype (87.7 mm x 78.4 mm).

terebratuliformis, *Ostrea cucullata* var. – Hanley, 1856a: 306 (footnote). No loc. stated. No type specimen has been located, and the species was unfigured. While it was listed as a synonym of the Indian Ocean *Saccostrea forskali* (Gmelin, 1791) by Inaba et al. (2004: 29–30), it is best considered a *nomen dubium* (Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

Unionidae

angustior, *Unio generosus* var. – Hanley & Theobald, 1872: 22, pl. 46, fig. 7. Pegu. Listed as a synonym of the Burmese *Lamellidens generosus* (Gould, 1847) in the freshwater mussel database: <http://mussel-project.ua.edu>. NHMUK 1968655, figured syntype (77.0 mm x 44.2 mm) (Johnson, 1971: 79, as “holotype”).

**ascia*, *Unio* – Hanley, 1856a: 385, 22 (pl. expl.), pl. 23, fig. 20, ex Benson ms. Penang. See also Prashad (1932b: 174–176). Haas (1910–1920) did not include this taxon. Haas (1969a: 99) listed it as *Contradens ascia*. Brandt (1974: 289–290, 392, pl. 24, fig. 49) recognized it as a subspecies, *Uniandra contradens ascia* (Hanley, 1856), noting the priority of *Uniandra* Haas, 1912, over *Contradens* Haas, 1913. This subspecies occurs in Penang, Malaysia. MCZ 17046, lectotype (57.3 mm x 34.3 mm) (selected by Johnson, 1956: 106, pl. 2, fig. 2).

aurea, *Unio indicus* var. – Hanley & Theobald, 1874: 43, pl. 107, fig. 4, ex G. B. Sowerby II ms. Nerbudda River. *Non Unio aureus* Lea, 1859. Presumed synonym of *Parreysia rajahensis* (Lea, 1841), which occurs the

Narmada Basin, west-central India (Simpson, 1900: 844; Preston, 1915: 169; Haas, 1969a: 124–125). Type material not found in NHMUK or Leeds Museum in 2012.

**corbis*, *Unio* – Hanley, 1856a: 386, 22 (pl. expl.), pl. 23, fig. 43, ex Benson ms; Hanley & Theobald, 1872: 22, pl. 45, fig. 10. Assam. *Parreysia corbis* (Hanley, 1856) from northeastern India (Prashad, 1932b: 176–177; Haas, 1969a: 121; Subba Rao, 1989; Ramakrishna & Dey, 2007: 295). Type material not found in NHMUK or Leeds Museum in 2012. Ramakrishna & Dey (2007: 295) erroneously stated: “Type location: Natural History Museum, London.”

cylindrica, *Unio marginalis* var. – Hanley & Theobald, 1872: 20, pl. 44, fig. 1. No locality provided. Synonym of *Lamellidens marginalis* (Lamarck, 1819) (Preston, 1915: 17), which is widespread in India, Sri Lanka, Bangladesh and Burma. Type material not found in NHMUK or Leeds Museum in 2012.

fragilis, *Unio corrugatus* var. – Hanley & Theobald, 1872: 21, pl. 45, fig. 4. Ex pisces? *Non U. fragilis* Rafinesque, 1820. Junior synonym of *Parreysia corrugata* (Müller, 1774) (Preston, 1915: 156), which is widespread in India, Bangladesh and Nepal. NHMUK 1968651, figured syntype (41.1 mm x 29.9 mm) (Johnson, 1971: 84, as “holotype” but the original description indicated more than one specimen).

**involutus*, *Unio* – Hanley, 1856a: 385, 22 (pl. expl.), pl. 23, fig. 19, ex Benson ms; Hanley & Theobald, 1872: 19, pl. 41, fig. 2. Assam. Haas (1969a) used *Radiatula* as a valid genus, but Haas (1969b), Subba Rao (1989) and Ramakrishna & Dey (2007: 319) ranked it as a subgenus of *Parreysia*. The first phylogenetic analysis including this group supports recognizing *Radiatula* as a valid genus separate from *Parreysia* (Whelan et al. 2011). Thus: *Radiatula involuta* (Hanley, 1856), which occurs in northeastern India and Bangladesh. NHMUK 1968656, holotype (43.0 mm x 29.5 mm) (Johnson, 1971: 86).

obesa, *Unio marginalis* var. – Hanley & Theobald, 1872: 20, pl. 43, fig. 3. Irawadi River, Birmah. *Non Unio obesa* Lea, 1831. Preston (1915: 176) placed it as a synonym of *Lamellidens marginalis* (Lamarck, 1819). However, it has been treated as a subspecies of *Lamellidens jenkinsianus* (Benson, 1862) from northeastern India, Bangladesh and Burma (Subba Rao, 1989: 167; Ramakrishna & Dey, 2007: 287–288). If it continues to be

retained as a subspecies, a replacement name will be needed. NHMUK 1907.12.30.46, holotype (Johnson, 1971: 89).

radula, *Unio* – Hanley, 1856a: 382, 22 (pl. expl.), pl. 23, fig. 41, ex Benson ms; Hanley & Theobald, 1870: 5, pl. 10, fig. 3 (holotype, as “Benson’s unique type”). Assam. *Non Unio radula* Say, 1829. Renamed *Nodularia (Radiatula) lima* Simpson, 1900: 820. Haas (1969a:112) also used *Radiatula lima* (Simpson, 1900). Subba Rao (1989: 190) and Ramakrishna & Dey (2007: 321) recognized *Parreysia (Radiatula) lima* (Simpson, 1900), but did not include *radula* in their synonymies. Assuming that *Radiatula* is a full genus (see remarks under *Unio involutus*), the valid name of this species would be *Radiatula lima* (Simpson, 1900), which occurs in northeastern India and Nepal. Ramakrishna & Dey (2007: 321) erroneously stated “Type location: USNM,” but the type material is that of Hanley, as Simpson merely renamed a junior homonym. Type material not found in NHMUK or Leeds Museum in 2012.

scobina, *Unio* – Hanley, 1856a: 382, 22 (pl. expl.), pl. 23, fig. 40, ex Benson ms; Hanley & Theobald, 1876: 22, pl. 46, figs. 2, 3. Assam. This taxon was overlooked by Haas (1969a) and Brandt (1974), but treated as a junior synonym of *Scabies crispata* (Gould, 1843) by Subba Rao (1989:193) and Ramakrishna & Dey (2007: 277–278), which is widespread from China through Indochina and India. Type material not found in NHMUK or Leeds Museum in 2012.

solida, *Unio corrugatus* var. – Hanley & Theobald, 1872: 21, pl. 45, fig. 2. Godavery River. *Non Unio solidus* Lea, 1838. Junior synonym of *Parreysia corrugata* (Müller, 1774), which is widespread in India, Bangladesh and Nepal (Haas, 1969a: 118). Type material not found in NHMUK or Leeds Museum in 2012.

tennentii, *Unio* – Hanley, in Hanley & Theobald, 1872: 22, pl. 45, figs. 7–9. This species was in part based on the image of *Mya corrugata* Müller, 1774, in Wood (1815: pl. 24, figs. 1–3), but it is also clear that Hanley had a Benson specimen from “British India”. It was not intended as a renaming of a homonym, as it has been interpreted by some workers. Preston (1915: 155) and Haas (1969a:118) both listed it as a junior synonym of *Parreysia corrugata* (Müller, 1774), which is widespread in India and Bangladesh as well as Nepal. Not listed by either Subba Rao (1989) or Ramakrishna

& Dey (2007). NHMUK 1907.12.30.58, syn-type (31.0 mm x 22.2 mm) (not mentioned by Johnson, 1971: 95).

trirostris, *Unio* – Hanley, 1863b: *Unio* pl. 2, fig. 9; Hanley & Theobald, 1870: 6, pl. 11, fig. 6. Hindostan (Hanley, 1863b); Moradabad (Hanley & Theobald, 1870). *Unio trirostris* “Musgrave” [Hanley’s photographer] was listed by Simpson (1900: 842; 1914: 1110), Preston (1915: 158–159), and Haas (1969a: 119) as a synonym of *Parreysia favidens* (Benson, 1862), which is widespread in India and Bangladesh, as well as Nepal. Not mentioned by either Subba Rao (1989) or Ramakrishna & Dey (2007). NHMUK 1907.12.30.45, holotype (63.0 mm x 43.1 mm) (Johnson, 1971: 96).

typica, *Unio marginalis* var. – Hanley & Theobald, 1872: 20, pl. 43, fig. 2. Moradabad, Pegu. Listed as synonym of *Lamellidens marginalis* (Lamarck, 1819), which is widespread in India, Sri Lanka, Bangladesh and Burma in the freshwater mussel database: <http://mussel-project.ua.edu>. Type material not found in NHMUK or Leeds Museum in 2012.

velaris, *Unio* – Hanley, 1856a: 385–386, 22 (pl. expl.), pl. 23, fig. 42, ex Benson ms. Bugis, Celebes; Assam, India. However, Hanley & Theobald (1876: 62) subsequently noted that the Indian locality was incorrect, overlooked by Prashad (1932b: 177–178). Haas (1910: 289; 1969a: 990) listed *velaris* as a junior synonym of *Physunio superbus* (Lea, 1843), which occurs in Thailand, the Malay Peninsula, Java and Sumatra. Brandt (1974: 294–295, 394, pl. 25, fig. 57) also did so, but tentatively. *Unio velaris* Hanley, 1856, is the type species of *Velunio* Haas, 1914, by original designation. Type material not found in NHMUK or Leeds Museum in 2012.

vulcanus, *Unio – Hanley, 1876: 606–607; Hanley & Theobald, 1876: 62, pl. 155, fig. 3. Birmah, vel Pegu; Hanley coll. Simpson (1900, 1914), Haas (1969a) and Brandt (1974) treated this species as *Parreysia vulcanus* (Hanley, 1876). Others have regarded it as a synonym of *Parreysia burmana* (Blanford, 1869), which occurs in the Irrawaddy and Salween rivers of Burma and adjacent Thailand, but possibly a separate species (Brandt, 1974: 280). NHMUK 1900.3.19.20, holotype (32.0 mm x 24.9 mm) (Johnson, 1971: 97).

walpolei, *Monocondylaea – Hanley, 1871: 587–588. Sawawak, Borneo; ex Geale coll.; Hanley coll. Haas (1910: pl. 41, fig. 7) and Simpson (1914: 1096) listed it as a valid

species, *Pseudodon walpolei* (Hanley, 1871), which occurs in Borneo (Haas, 1969a: 131). MCZ 175577, lectotype (43.7 mm x 27.9 mm) (selected by Johnson, 1948: 48–50, pl. 2, fig. 2); NHMUK 1871.7.11.1, paralectotype (51.0 mm x 30.3 mm) (Johnson, 1971: 97).

zonata, *Unio marginalis* var. – Hanley & Theobald, 1872: 20, pl. 44, fig. 2. Belgaum, Deccan, India. Preston (1915: 177) retained this as a variety of *Lamellidens marginalis* (Lamarck, 1819). However, it is now regarded as a synonym of *L. scutum* (G. B. Sowerby II, 1868), which occurs in Tenasserim, Burma (Subba Rao, 1989: 169). NHMUK 1907.10.28.252, figured syntype (57.8 mm x 32.4 mm) (Johnson, 1971: 97, as “holotype”).

bhamaoensis, *Unio* – “Hanley & Theobald”, 1876: 62. Spelling error for *Unio bhamoensis* Theobald, 1873.

birmanus, *Unio* – “Hanley & Theobald”, 1870: 19. Spelling error for *Unio burmanus* Blandford, 1869.

candaharicus, *Unio marginalis* – “Hanley & Theobald”, 1872: 20, pl. 43, fig. 4. This species has been listed by some authors as a Hanley & Theobald name, but it was proposed by Hutton (1850: 660).

inornatus, *Unio* – “Hanley ms”. In his section of the *Conchologia Iconica* monograph on *Unio*, Reeve (1865: pl. 29, fig. 147) indicated that this species was a Hanley manuscript name; the Errata to this monograph by G. B. Sowerby II (1868), indicated that this species name should have been credited to Lea, 1856. It was additionally a misidentification, as the figured specimen actually was *Contradens contradens* (Lea, 1838).

mandelayanus, *Unio* – Hanley & Theobald, 1876: 62. Spelling error for *Unio mandelayensis* Theobald, 1873.

Mulleriidae

bensonianus, *Mycetopus* – Hanley & Theobald, 1876: v. *Nomen novum pro Anodonta soleniformis* Benson, 1836, *non Unio soleniformis* Lea, 1831, and *non Mycetopoda soleniformis* d’Orbigny, 1835. An unnecessary replacement name, since the replacement name *Margaron* (*Unio*) *bensonii* Lea, 1870, had already been supplied for Benson’s name. This Indian species is now known as *Solenaia soleniformis* (Benson, 1836), the species taxa involved

all now referred to different genera and the replacement names not having gained acceptance (Bogan, in press; ICZN Code Art. 59.3).

Lucinidae

philippinarum, *Lucina* – “Hanley”. This species was described by Reeve (1850: pl. 4, fig. 18a, b) and credited to “Hanley, Species of Shells”, but it did not appear in Hanley’s text until 1856 (Hanley, 1856a: 348), in which he cited Reeve’s monograph. This Reeve species, ex Hanley ms, is now regarded as a synonym of *Austriella corrugata* (Deshayes, 1843) (Glover et al., 2008: 27–29).

Astartidae

latisulca, *Crassina* – Hanley, 1843b: 87 (footnote); 1844g: pl. 14, fig. 35; 1846c: 9 (pl. expl.). North America. Synonym of northwestern Atlantic *Astarte undata* Gould, 1841 (E. A. Smith, 1881: 206; Dall, 1903: 938). Leeds Museum 1957.173.602, possible syntype (33.7 mm x 28.6 mm (same size as Hanley’s measurements, but with more ribs than the figured specimen)).

Chamidae

bonanni, *Chama arcinella* – Hanley, 1885b: 292. “Brazil? Zanzibar?”; Hanley coll., ex Belcher coll. Hanley was ambivalent as to whether this was a variety of *C. arcinella* or a separate species, so he listed it both ways. Indeterminate (Nicol, 1952: 810, as “*C. bonnanii*”). Type material not found in NHMUK or Leeds Museum in 2012.

Cyrenoididae

Cyrenoidea – Hanley, 1846c: 10 (pl. expl.). Invalid emendation of *Cyrenoida* Joannis, 1835.

Galeommatidae

Galeomna – “Hanley”, 1843b: 59. Error pro *Galeomma* Turton, 1825.

Lasaeidae

coquimbensis, *Montacuta – Hanley, 1857: 340. Coquimbo, Chile; 6 fms. [11 m], fine sand; H. Cuming. *Mysella coquimbensis* (Hanley,

1857) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1967992, five syntypes.

***mactroides, Pythina** – Hanley, 1857: 340. Cape of Good Hope, South Africa. *Pythina mactroides* Hanley, 1857 (Barnard, 1964: 486–487); *Melliteryx mactroides* (Hanley, 1857) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1967994, four syntypes.

***nuculoides, Pythina** – Hanley, 1857: 341. Huacna, Society Islands; H. Cuming. *Lionelita nuculoides* (Hanley, 1857) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 20120170, 1 syntype (10.2 mm x 8.2 mm).

***tellinoides, Kellia** – Hanley, 1857: 340. Baclayon, Bohol, Philippine Islands; under stones; H. Cuming collection. NHMUK 1967993, three syntypes.

Cyrenidae

cochinensis, Cyrena – Hanley, 1859a: 543; 1863b: pl. 7, fig. 2. Cochin [Kochi], India; Hanley coll., from M'Andrew. *Villorita cyprinoides* var. *cochinensis* (Hanley, 1856) (Satyamurti, 1960: 151–152); synonym of the southwestern Indian *Villorita cyprinoides* (Gray, 1825) (Huber, pers. comm., 13 May 2012). Type material not found in NHMUK or Leeds Museum in 2012. MCZ 119016 (Madras Coast, India), is catalogued as “cotypes” but these are only specimens “identified by Hanley” (Prime, 1895: 19), neither specified as from Cochin or from the M'Andrew collection (Johnson, 1959: 447, as “idiotypes”).

***iravadica, Corbicula** – Hanley & Theobald, 1876: 62, pl. 155, fig. 8, ex Blanford ms; Blanford, 1881: 221. Irrawaddy River, Pegu and Ava, Myanmar. A valid species of *Corbicula* (Prashad, 1928: 25–26, pl. 4, figs. 7–10; Brandt, 1974: 323, 400, pl. 28, fig. 91). NHMUK 1907.12.30.406, probable holotype (11.8 mm x 10.0 mm).

***philippinarum, Cyrena** – Hanley, 1844g: 10 (pl. expl.), pl. 14, fig. 60; 1845b: 159–160; 1856a: 351–352. Philippine Islands; Cuming & Hanley colls. *Batissa philippinarum* (Hanley, 1844) (Prime, 1869: 139). NHMUK 1842.5.10.1513, figured syntype (106.3 mm x 119.3 mm).

pisum, Corbicula – Hanley & Theobald, 1876: 62, *nomen nudum*, in synonymy with *C. iravadica* Hanley & Theobald, 1876, ex Blanford ms.

***placens, Cyrena** – Hanley, 1844g: 10 (pl. expl.), pl. 14, fig. 52; 1845b: 160; 1856a: 352. Honduras (Hanley, 1856a); Hanley coll. &

“Jardin des Plantes” [MNHN, but not extant, V. Héros, pers. commun., 15 Oct. 2012]. As *Cyanocyclas placens* (Hanley, 1844), probably from South America (Simone, 2006: 298: fig. 1043, syntype); *Polymesoda placens* (Hanley, 1844) from Central America (Abbott & Dance, 1982: 352). NHMUK 1907.10.28.3, syntype (37.5 mm x 42.2 mm).

***radiata, Cyrena** – Hanley, 1845b: 159; 1863b: pl. 7, fig. 4. Central America; Cuming, Hanley & G. B. Sowerby II colls.; probably collected by H. Cuming. The tropical eastern Pacific *Polymesoda radiata* (Hanley, 1845) (Coan & Valentich-Scott, 2012: 472–474). NHMUK 1907.10.28.5, syntype; NHMUK 1992127, 2 syntypes; MCZ 176946, 2 syntypes (36.5 mm x 33.0 mm; 34.5 mm x 33.4 mm) (Johnson, 1959: 468). MCZ 176947 was catalogued as “cotypes” or “idiotypes” but this lot appears to be merely specimens later “identified by Hanley” (Prime, 1895: 21).

sordida, Cyrena – Hanley, 1844g: 10 (pl. expl.), pl. 14, fig. 51; 1845b: 159; 1856a: 352. North America (1845b); “Florida?” (1856a); Hanley coll. Prime (1869: 148) stated that this species was from Central America. M. Huber (pers. comm., 20 Dec. 2011), following notes by J. P. E. Morrison, considers this to be a synonym of the western Atlantic *Polymesoda carolinina* (Bosc, 1801). *Cyrena sordida* Hanley, 1844, is the type species of *Americana* Clessin, 1879, by the subsequent designation of Morrison (1959). NHMUK 1907.10.28.4, holotype (36.3 mm x 39.1 mm).

tennentii, Cyrena – Hanley, 1858a: 23–24; 1859c: 237. Ariho River, Taprobana [Sri Lanka]; “below the great stone dam”. *Cyrena tennentii* Hanley, 1858 (Preston, 1915: 329; Prashad, 1921: 142, pl. 20, fig. 10). Synonym of *Geloina coaxans* (Gmelin, 1791) (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

cashmirensis, Corbicula – “Hanley & Theobald”, 1875: 55. Spelling error for *C. cashmiriensis* Deshayes, 1855 (Prashad, 1928: 20).

Glauconomidae

***rugosa, Glaucanome** – Hanley, 1843f: 2 (Syst. List, as “rogosus”), 3 (pl. expl., as *rugosus*); 1843g: pl. 10, fig. 24; 1846c: 3 (pl. expl., as *rugosus*); 1856a: 337, as *rugosa*; Manila, Philippine Islands. This species be-

came available when plate 10 was published, providing a figure to go with the plate explanation published earlier. *Glaucanome rugosa* Hanley, 1843, from the Philippine Islands and Australia (Huber, 2010: 435). NHMUK 20110079, three possible syntypes.

curta, *Glaucanome* – “Hanley”. Reeve, 1844: pl. 1, fig. 7; Hanley, 1844g: pl. 15, fig. 41 (without caption); 1846c: 10 (pl. expl.); 1856a: 337. Philippine Islands (1856a). Hanley (1856a: 337) indicated this as being his species, but Reeve’s account in the *Conchologia Iconica* was published in April 1844, well before Hanley’s 1846 plate caption appeared.

virens, *Glaucanome* – “Hanley”, 1844c: 18. Listed by Norris & Dance (2002: 375) as being a Hanley species, this is *Solen virens* Linnaeus, 1767, as indicated by Hanley.

Tellinidae

****acuminatus*, *Tellinides*** – Hanley, 1843b: 73–74; 1843f: 8 (pl. expl.); 1843g: pl. 13, fig. 53; 1846a: 294–295, 331, pl. 60, fig. 141, as *Tellina* (*Tellinides*) *acuminata*. No loc. stated; British Museum. Réunion (Deshayes, 1863: 11). *Omala* (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 20120175, syntype (figured valve is broken; other valve 47.8 mm x 27.7 mm).

****ala*, *Tellina*** – Hanley, 1845a: 165; 1846a: 311, 331, pl. 60, figs. 137, 146; 1859c: 236. “Ceylon?” [Sri Lanka]; Cuming, Hanley & Metcalfe colls. Dey (2006: 69–70, pl. 32, fig. 1, pl. 35, fig. 6, as “*Macoma* (*Psammotreta*) *ala* (Hanley, 1844)”); Hylleberg & Kilburn (2002: 68 as “*Arcopagia*”). *Tellina*, s.l. (M. Huber, pers. comm., 13 May 2012). Type material not found in NHMUK or Leeds Museum in 2012.

****ancilla*, *Tellina*** – Hanley, 1844f: 148; 1846a: 304, 331, pl. 58, fig. 94. Lord Hood’s Island [Marutea, Tuamotus]; fine coral sand; H. Cuming. NHMUK 20120176/1, lectotype (selected by Salisbury, 1934: pl. 11, fig. 5, as “holotype”) (24.2 mm x 20.2 mm); NHMUK 20120176/2, paralectotype. *Tellina* s.l., from Philippine Islands (M. Huber, pers. comm., 2 Sept. 2012).

****asperrima*, *Tellina*** – Hanley, 1844d: 59–60; 1846a: 226–227, 331, pl. 60, fig. 135. Sual, Pangasinan, Luzon, Philippine Islands; 6 fms. [11 m], sandy mud; H. Cuming. The Indo-Pacific *Tellina* (*Tellinella*) *asperrima* Hanley,

1844 (Poppe & Langleit, 2011: pl. 1154, figs. 5–7). Synonym of *Scutarcopagia pulcherrima* (G. B. Sowerby I, 1825) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1996472, possible syntype (this lot has two specimens, while the original description refers to the “unique specimen,” so one of the two specimens may have been subsequently added to this lot) (Higo et al., 2001: 169, fig. B916).

****assimilis*, *Tellina*** – Hanley, 1844f: 144; 1846a: 302–303, 331, pl. 58, fig. 95. Luzon [Philippine Islands]; 6 fms. [11 m], sandy mud; H. Cuming. Xu et al. (2008: 193, fig. 600), as *Exotica assimilis* (Hanley, 1844). *Tellina*, s.l. (Huber, pers. comm., May 13, 2012). Type material not found in NHMUK or Leeds Museum in 2012. NHMUK 1874.12.11.322 was figured by G. B. Sowerby II (1867: pl. 34, fig. 191), from the Lombe Taylor collection is conspecific, but is not a type specimen.

****aurora*, *Tellina*** – Hanley, 1844f: 147; 1846a: 301, 331, pl. 58, fig. 76. Panama; 10 fms. [18 m], soft sandy mud; H. Cuming. The tropical eastern Pacific *Psammotreta* (*Psammotreta*) *aurora* (Hanley, 1844) (Coan & Valentich-Scott, 2012: 612–613). *Tellina aurora* Hanley, 1844, is the type species of *Psammotreta* Dall, 1900, by original designation. NHMNK 1990045/1, lectotype (27.6 mm x 17.2 mm) (selected by Salisbury, 1934: pl. 11, fig. 4); NHMUK 1990045/2, paralectotype; Leeds Museum 1957.173.172, two possible paralectotypes (larger is 30.3 mm x 18.3 mm).

****bruguieri*, *Tellina*** – Hanley, 1844f: 142; 1846a: 321–322, 332, pl. 62, fig. 192. Ilo-Ilo, Panhay [Philippine Islands]; hard sand; H. Cuming. In 1846a, Hanley also cited Bruguière (1797: pl. 231, fig. 2). The western Pacific *Macalia bruguieri* (Hanley, 1844) (Hylleberg & Kilburn, 2002: 68; 2003: 199; Wang, 2004b: 284, pl. 156, fig. N; Poppe & Langleit, 2011: pl. 1165, fig. 2). *Tellina bruguieri* Hanley, 1844, is the type species of both *Macalia* H. Adams, 1861, by monotypy, and of *Tellinungula* Römer, 1873, by monotypy, so that the latter is an objective junior synonym of the former. NHMUK 1976133, two syntypes (figured syntype 43.0 mm x 37.2 mm) (Higo et al., 2001: 171, fig. B1003); Leeds Museum 1957.173.24642, one syntype (37.9 mm x 33.7 mm).

****carnicolor*, *Tellina*** – Hanley, 1846a: 263, pl. 56, fig. 15, *nom. nov. pro T. incarnata* Hanley, 1844, *non T. incarnata* Linnaeus. The Indo-Pacific *Clathrotellina carnicolor* (Hanley, 1846) (Higo et al., 2001: 170, fig.

- B938, syntype; Hylleberg & Kilburn, 2003: 198). NHMUK 1874.12.11.338, figured syntype (19.2 x 16.3 mm); Leeds Museum 1957.173.1864, two possible syntypes (33.4 mm x 18.8 mm; 31.7 mm x 19.3 mm) (the size of the Leeds specimens is large for this species).
- ***casta, *Tellina*** – Hanley, 1844d: 63; 1846a: 253, 330, pl. 56, fig. 9. Singapore; sandy mud; H. Cuming. The Indo-Pacific *Arcopella casta* (Hanley, 1844) (Lyngé, 1909: 193; Hylleberg & Kilburn, 2003: 197–198; Wang, 2004b: 280, pl. 154, fig. G), or *Arcopagia casta* (Hanley, 1844) (Dey, 2006: 57–58, pl. 24, figs. 2, 3, pl. 27, fig. 5). *Abranda* (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 1976123, 4 syntypes (seven valves; largest valve is the figured specimen, 12.4 mm x 9.9 mm).
- ***chinensis, *Tellina*** – Hanley, 1845a: 165; 1846a: 326–327, 330, pl. 56, fig. 18. China; British Museum coll. *Tellinides chinensis* (Hanley, 1845) (Wang, 2004b: 277, pl. 152, fig. I, as “1844”); however, M. Huber (pers. comm. 2 Sept. 2012) concluded that its locality and status are unresolved, and it probably belongs close to *Macoma* because of its lack of lateral teeth. This would leave the Chinese tellinid without an available name. NHMUK 1843.11.23.60, holotype (24.6 mm x 16.6 mm).
- ***columbiensis, *Tellina*** – Hanley, 1844d: 71; 1846a: 307, 333, pl. 65, fig. 246. Monte Christi, West Colombia [Ecuador]; 12 fms. [22 m], sandy mud; H. Cuming. The tropical eastern Pacific *Psammotreta (Psammotreta) columbiensis* (Hanley, 1844) (Coan & Valentich-Scott, 2012: 613–614). *Tellina columbiensis* Hanley, 1844, is the type species of *Ardeamya* Olson, 1961, by original designation. NHMUK 1966548, 2 syntypes (two valves that do not match; 51.2 mm x 28.1 mm and 53.4 mm x 28.9 mm); Leeds Museum 1957.173.173, one syntype (56.1 mm x 30.9 mm). Hanley (1844d) gave the size of this species as 3 inches by 1.7 inches [= c. 75 mm x 43 mm], which is somewhat larger than any of these three syntypes.
- ***conspicua, *Tellina*** – Hanley, 1846a: 293, pl. 58, fig. 100. Loc. not stated; Cuming coll. *Tellinides conspicuus* (Hanley, 1846) from Indonesia (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.
- ***corbuloides, *Tellina*** – Hanley, 1844d: 70; 1846a: 280–281, 330, pl. 57, figs. 50, 57. Catbalonga, Samar [Philippine Islands]; 10 fms. [18 m], soft mud; H. Cuming. *Non T. corbuloides* F. A. Römer, 1836. The western Indo-Pacific *Tellina (Angulus) corbuloides* Hanley, 1844 (Lyngé, 1909: 201–202), but this species requires the use of a synonym or renaming if it is recognized as valid. NHMUK 1976130, two syntypes (specimen figured in fig. 57 is 32.3 mm x 21.7 mm).
- ***culter, *Tellina*** – Hanley, 1844d: 69–70; 1846a: 279–280, 330, pl. 57, fig. 51. Cagayan, Misamis, Mindanao [Philippine Islands]; 25 fms. [46 m], sandy mud; H. Cuming. The western Pacific *Moerella culter* (Hanley, 1844) (Hylleberg & Kilburn, 2003: 200; Wang, 2004b: 285, pl. 156, fig. B, with *Moerella* as full genus; Poppe & Langleit, 2011: pl. 1158, fig. 10). Not a *Moerella*, but rather *Tellina*, s.l. (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 2012084, three possible syntypes.
- ***cumingii, *Tellina*** – Hanley, 1844d: 59; 1846a: 223, 331, pl. 58, fig. 72. Guacomayo, Central America [possibly on eastern shore of the Golfo de Montijo, Panama – Coan, 2011]; coral sand; H. Cuming. The tropical eastern Pacific *Tellina (Tellinella) cumingii* Hanley, 1844 (Coan & Valentich-Scott, 2012: 656, 658). NHMUK 20120177, syntype (35.1 mm x 15.7 mm).
- ***cuspis, *Tellina*** – Hanley, 1844d: 72; 1846a: 309–310, 331, pl. 59, fig. 125. Loc. unknown; Cuming, Walton & Metcalfe colls. A valid *Tellina*, s.l., from Indonesia and/or Philippines (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 20120178, figured syntype (33.3 mm x 22.2 mm).
- ***cycladiformis, *Tellina*** – Hanley, 1844d: 70; 1846a: 277–278, 330, pl. 57, fig. 61. San Nicolas, Zebu [Philippine Islands]; H. Cuming. *Macoma cycladiformis* (Hanley, 1844) from the Philippine Islands (Poppe & Langleit, 2011: pl. 1165, figs. 5, 6). *Pinguitellina cycladiformis* (Hanley, 1844) from the Philippine Islands (M. Huber, pers. comm., 13 May 2012). Type material not found in NHMUK or Leeds Museum in 2012.
- ***cygnus, *Tellina*** – Hanley, 1844f: 144; 1846a: 310–311, 331, pl. 59, fig. 122. Bias, Negros [Philippine Islands]; 7 fms. [13 m], coral sand; H. Cuming. The western Indo-Pacific *Tellina cygnus* Hanley, 1844 (Scott, 1994: 86–87, pl. 12, fig. 1A; Hylleberg & Kilburn, 2003: 202). NHMUK 1874.12.11.373, syntype (13.4 mm x 8.7 mm) (Simone & Wilkinson, 2008: 175, fig. 53–55, as the “probable holotype/syntype” of *Pinguimacoma cygnus*).
- ***cyrenoidea, *Tellina*** – Hanley, 1844d: 64; 1846a: 258, 330, pl. 56, fig. 31. St. Nicolas, Zebu, Philippine Islands; low water, sandy

mud; H. Cuming. The Indo-Pacific *Strigilla* (*Aeretica*) *cyrenoidea* (Hanley, 1844) (M. Huber, pers. comm., 13 May 2012). NHMUK 20120179, three syntypes (figured syntype is 16.6 mm x 15.5 mm). Salisbury (1934: pl. 13, fig. 2) illustrated the “holotype,” but his figure is of another species.

deshayesii, *Tellina – Hanley, 1844f: 148; 1846a: 223, 330, pl. 57, fig. 66 (as “96” on p. 330). “Red Sea?”; Cuming & Deshayes colls. *Dallitellina deshayesii* (Hanley, 1844) from southeast Asia (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 20120180, two syntypes (figured syntype, 38.9 mm x 14.9 mm; other syntype, 44.1 mm x 16.4 mm).

diana, *Tellina – Hanley, 1844f: 147–148. “Java?”; Hanley coll. Synonym of *Psammacoma candida* (Lamarck, 1818), and from China, not Java (M. Huber, pers. comm., 2 Sept. 2012).

discus, *Tellina – Hanley, 1844d: 63–64; 1846a: 257–258, 333, pl. 64, figs. 228, 232. Mindanao [Philippine Islands]; coarse sand, on reefs; H. Cuming. *Cyclotellina discus* (Hanley, 1844) from the Philippine Islands (Poppe & Langleit, 2011: pl. 1162, figs. 1, 2). Type material not found in NHMUK or Leeds Museum in 2012.

dombei, *Tellina – Hanley, 1844f: 144; 1846a: 323–324, 332, pl. 62, fig. 182, 333, pl. 64, fig. 222. Panama; 12 fms. [22 m], sandy mud; H. Cuming. The tropical eastern Pacific *Psammotreta* (*Leporimetis*) *dombei* (Hanley, 1844) (Coan & Valentich-Scott, 2012: 617–618). The species name was emended to what would have been the more correct *dombeyi* by some authors, but Hanley’s original spelling has to be maintained (ICZN Code Art. 32.5). NHMUK 1966550, four syntypes (one specimen was figured by G. B. Sowerby II, 1867: pl. 30, fig. 169).

eburnea, *Tellina – Hanley, 1844d: 61; 1846a: 241–242, 331, pl. 58, fig. 91. Tumbes, Peru; 5 fms. [9 m], soft sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Eurytellina*) *eburnea* Hanley, 1844 (Coan & Valentich-Scott, 2012: 635–636). Type material not found in NHMUK or Leeds Museum in 2012.

elongata, *Tellina* – Hanley, 1844f: 144; 1846a: 302, 332, pl. 62, fig. 199. [Golfo de] “Chiriqui” [Chiriqui], West Colombia [Panama]; 3 fms. [5.5 m], sand; H. Cuming. *Non Tellinites elongatus* Schlothem, 1820 (under the ICZN Code Art. 11.5, *Tellinites* is equivalent to *Tellina*). Synonym of *Macoma* (*Psammacoma*) *elytrum* Keen, 1958 (Coan & Valentich-Scott,

2012: 605–606). NHMUK 1874.12.11.354, lectotype (selected by Myers, et al., 1983a: 64, figs. 2–3) (51.0 mm x 27.2 mm); NHMUK 198331, 198332, two paralectotypes (Myers, et al., 1983a: fig. 6). Myers, et al. (1983b: 88) stated that: “*Tellina elongata* Hanley, 1844 is a primary homonym of *T. elongata* Dillwyn, 1823, and therefore must be permanently rejected (ICZN Article 59A)” [referring to the pre-1985 Code; comparable wording is in ICZN Articles 23.9.5 and 57.2 of the 2000 Code]. However, Keen (1958) explicitly did not propose a replacement name but rather a new species in light of such homonymy (Coan & Petit, 2006: 114–115).

fabricii, *Tellina* – Hanley, 1846a: 318, 331, pl. 59, fig. 112. *Nom. nov. pro Tellina fragilis* (Fabricius, 1780: 413 – originally as *Venus*), *non T. fragilis* Linnaeus, 1758. Northern Seas. Synonym of the Arctic and boreal *Macoma balthica* (Linnaeus, 1758) (Coan et al., 2000: 417). Since this was a replacement name, the type material would be the specimen(s) that Fabricius had.

felix, *Tellina – Hanley, 1844d: 71; 1846a: 281, 330, pl. 57 (as “58” on p. 281), fig. 52. Panama; 6–10 fms. [11–18 m], sandy mud; H. Cuming. *Tellina* (*Moerella*) *felix* Hanley, 1844 (Coan & Valentich-Scott, 2012: 648–650). Type material not found in NHMUK or Leeds Museum in 2012.

fimbriata, *Tellina – Hanley, 1844f: 149; 1846a: 262, pl. 60, fig. 132. Loc. unknown; Cuming coll. *Arcopagia fimbriata* (Hanley, 1844) from Réunion and Mauritius (Drivas & Jay, 1988: 144, pl. 57, fig. 6); however, Huber (pers. comm., 2 Sept. 2012) concluded that this was referable to *Serratina*. NHMUK 20120181, two syntypes (smaller may be that figured by Hanley in fig. 132, 34.7 mm x 29.8 mm; larger syntype was figured by G. B. Sowerby II, 1866: 17, pl. 30, fig. 166, 40.3 mm x 32.8 mm).

formosa, *Tellina – Hanley, 1844f: 142; 1846a: 305, 331, pl. 58, fig. 83. Daleguete, Zebu [Philippine Islands]; H. Cuming. *Jactellina formosa* (Hanley, 1844) (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 20120182, figured holotype (13.3 mm x 10.3 mm).

frigida, *Tellina* – Hanley, 1844f: 143–144; 1846a: 327, 331, pl. 59, fig. 119. Kamchatka [Russia]; Petit de la Saussaye & Hanley colls. Synonym of *Macoma calcarea* (Gmelin, 1791) (Coan et al., 2000: 405, 409). NHMUK 1907.10.28.37, lectotype (selected by Coan, 1971: 27, pl. 7, fig. 34); NHMUK 1907.10.28.38, paralectotype).

- ***gelida, *Tellina*** – Hanley, 1846a: 226, unfigured; Belcher coll. No loc. given. Type material not found in NHMUK or Leeds Museum in 2012. Species not identifiable, and best treated as a *nomen dubium* (M. Huber, pers. comm., 2 Sept. 2012).
- ***gouldii, *Tellina*** – Hanley, 1846a: 272, 330, pl. 56, fig. 26. West Indies; Guilding. The western Atlantic *Tellina* (*Arorylus*) *gouldii* Hanley, 1846 (Boss, 1966: 270–272, pl. 139, fig. 4, pl. 142, fig. 4). Type material not found in NHMUK or Leeds Museum in 2012.
- ***grandis, *Tellina*** – Hanley, 1844f: 141–142; 1846a: 327, 333, pl. 65, fig. 247. Tumbes [Tumbes], Peru; H. Cuming. The tropical eastern Pacific *Psammotreta* (*Psammotreta*) *grandis* (Hanley, 1844) (Coan & Valentich-Scott, 2012: 613–614). NHMUK 20120183, three syntypes (1 paired valves, and two unmatched valves, one labeled “measured syntype,” 82.7 mm x 62.2 mm); Leeds Museum 1957.173.175, two syntypes (two unmatched valves, 74.3 mm x 52.7 mm; 70.4 mm x 52.9 mm).
- ***gubernaculum, *Tellina*** – Hanley, 1844f: 142; 1846a: 325, 332, pl. 62, fig. 186. “Real Llejos, Central America; 7 fms. [13 m], sandy mud; H. Cuming”. These specimens actually came from India (Coan & Valentich-Scott, 2012: 614–615). It also occurs in Vietnam (Hylleberg & Kilburn, 2003: 199, as *Macoma*; 201, as *Psammotreta*). Referrable to *Psammacoma* (M. Huber, pers. comm., 2 Sept. 2012). Two lots were identified by S. Whybrow in 1976 as possible syntypes, although they do not match Hanley’s illustration: NHMUK 1976126, 2 possible syntypes; NHMUK 1976127, 1 possible syntype.
- ***gouldingii, *Tellina*** – Hanley, 1844d: 60; 1846a: 230, 330, pl. 56, fig. 1. West Indies; Metcalfe & Walton colls. The western Atlantic *Tellina* (*Eurytellina*) *gouldingii* Hanley, 1844 (Boss, 1966: 293–295, pl. 149, fig. 2, pl. 151, figs. 1, 2). Type material not found in NHMUK or Leeds Museum in 2012.
- ***hiberna, *Tellina*** – Hanley, 1844f: 148; 1846a: 282, 330, pl. 57, fig. 53. Panama & Guayaquil [Guayas, Ecuador]; 6–11 fms. [11–20 m]; sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Eurytellina*) *hiberna* Hanley, 1844 (Coan & Valentich-Scott, 2012: 635–636). NHMUK 1982296/2, syntype (figured by Salisbury, 1934: pl. 13, figs. 7–9, as the “holotype” but the original description makes clear that the species was based on more than one specimen, per ICZN Code Article 74.5) (19.1 mm x 10.8 mm); NHMUK 1982296/1, 1982296/3–5, four syntypes.
- ***hilaris, *Tellina*** – Hanley, 1844f: 140; 1846a: 281–282, 330, pl. 57, fig. 54. Loc. unknown (“I suspect it comes to us from the Philippines”); Cuming coll. NHMUK 1984048, three syntypes (figured syntype 15.7 mm x 9.3 mm).
- ***imbellis, *Tellina*** – Hanley, 1844d: 143; 1846a: 276–277, pl. 60, fig. 155. Loc. unknown; Cuming coll. *Tellinota imbellis* (Hanley, 1844) from Australia (M. Huber, pers. comm., 20 Dec. 2011); figured as *Tellina imbellis* Hanley, 1844 by Lamprell & Whitehead (1992: pl. 46, fig. 332). Type material not found in NHMUK or Leeds Museum in 2012.
- incarnata, *Tellina*** – Hanley, 1844d: 68; 1846a: 263, 330, pl. 56, fig. 15. *Non Tellina incarnata* Linnaeus, 1758. Renamed as *T. carnicolor* Hanley, 1846a (see above). San Nicolas, Zebu [Philippine Islands]; low water, sandy mud; H. Cuming. NHMUK 1874.12.11.338, figured syntype (Higo et al., 2001: 170, fig. B938); Leeds Museum 1957.173.1864, two possible syntypes.
- inaequalis, *Tellina*** – Hanley, 1844d: 71; 1846a: 278–279, pl. 57, fig. 44; 1859c: 236. *Non Tellina inaequalis* J. De C. Sowerby, 1824. Ceylon [Sri Lanka]; Cuming coll. Synonym of the western Indo-Pacific *Quadrans gargadia* (Linnaeus, 1758) (Lyngø, 1909: 196; Dey, 2006: 40). Type material not found in NHMUK or Leeds Museum in 2012.
- ***inornata, *Tellina*** – Hanley, 1844f: 144; 1846a: 315–316, 331, pl. 59, fig. 123. Concepción [Concepción], Chile; 6 fms. [11 m], soft mud; H. Cuming. The Chilean *Macoma* (*Psammacoma*) *inornata* (Hanley, 1844) (Ramorino, 1968: 220–223, pl. 3, fig. 5, pl. 10, figs. 1, 2). NHMUK 1874.12.11.358, two syntypes (two non-matching valves, one broken, other 27.1 mm x 20.2 mm).
- ***insculpta, *Tellina*** – Hanley, 1844d: 70; 1846a: 289, 331, pl. 60, fig. 136. [Golfo de] Chiriqui, West Colombia [Panama]; 3 fms. [5.5 m], sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Phyllodella*) *insculpta* Hanley, 1844 (Coan & Valentich-Scott, 2012: 653–654). *Tellina insculpta* Hanley, 1844, is the type species of *Phyllodella* Hertlein & Strong, 1949, by monotypy. NHMUK 1966537, possible syntype (42.4 mm x 21.5 mm). Hanley’s description refers to “This unique and elegant shell,” suggesting that it was based on one specimen, but the published dimensions (2 inches x 1 inch, c. 50 mm x 25 mm), are slightly larger than the NHMUK specimen.

irus, *Tellina – Hanley, 1845a: 166; 1846a: 319, 331, pl. 60, fig. 145. Loc. unknown; Cuming & Walton colls. The western Pacific *Heteromacoma irus* (Hanley, 1845) (Matsukuma, 2000: 982–983; Wang, 2004b: 284, pl. 156, fig. N, as “1844”). *Tellina irus* Hanley, 1845, is the type species of *Heteromacoma* Habe, 1952, by original designation. NHMUK 1966483/1, syntype (figured by Salisbury, 1934: 85, pl. 12, figs. 7, 8, as the “holotype” but the original description makes clear that there were two or more syntypes, from two collections) (26.9 mm x 21.1 mm); Higo et al., 2001: 171, fig. B984); NHMUK 1966483/2, syntype (34.4 mm x 27.5 mm). Salisbury (1934: 85) gave the type locality as “Guinea,” in error.

jubar, Tellina – Hanley, 1844d: 60; 1846a: 229, 332, pl. 63, fig. 214. Loc. unknown; Cuming & Sowerby colls. Synonym of the Indo-Pacific *Tellinella virgata* (Linnaeus, 1758) (Prashad, 1932a: 178–180). NHMUK 20120213, two syntypes (68.0 mm x 46.2 mm; 66.9 mm x 42.7 mm) (labeled as coming from “Montebello Ids. W. Australia” and “Swan River”). Hanley’s original description stated: “Two specimens only of this rare Tellen are as yet known.” MCZ 228033 (2 valves, 53.0 mm x 37.0 mm) was catalogued as “cotypes” from the Philippine Islands (Cuming coll.), but are probably only specimens later identified by Hanley (“idiotypes”).

juvenilis, *Tellina – Hanley, 1844f: 140–141; 1846a: 288, 330, pl. 57, fig. 63. Philippine Islands; H. Cuming. NHMUK 1986049, syntype (figured by Salisbury, 1934: 88, pl. 12, fig. 1, as “holotype,” but this was not a valid lectotype selection under either ICZN Code Art. 74.5 or 74.6) (15.1 mm x 11.7 mm).

laceridens, *Tellina – Hanley, 1844d: 61–62; 1846a: 243–244, 332, pl. 61, figs. 168, 176, 333, pl. 66, fig. 258. Tumbes, Peru; 3 fms. [5.5 m], soft sandy mud; H. Cuming. The tropical eastern Pacific *Tellina (Eurytellina) laceridens* Hanley, 1844 (Coan & Valentich-Scott, 2012: 637–638). NHMUK 20120230, two syntypes. NHMUK 1874.12.11.254 was labeled as a probable syntype, but is from the Lombe Taylor collection, and may instead represent the specimen later figured by G. B. Sowerby II (M. Huber, pers. comm. 2 Sept. 2012).

lilium, *Tellina – Hanley, 1844f: 147; 1846a: 303, 331, pl. 58, fig. 85. Burias, low water, sandy mud; Negros, 7 fms. [13 m], coral sand; [both Philippine Islands]; H. Cuming. *Semelangulus lilium* (Hanley, 1844) (Hylleberg & Kilburn, 2003: 201); a valid Indo-Pacific

Tellina, s.l. (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 20120185, syntype (20.0 mm x 12.5 mm).

listeri, Tellina – Hanley, 1844d: 69; 1846a: 278, 333, pl. 65, fig. 251. Senegal; Cuming & Hanley colls. Hanley (1844d: 69; 1846a: 278) also cited Lister (1687: fig. 235). *Non T. listeri* Röding, 1798. Renamed *T. senegambiensis* Salisbury, 1934 (p. 85), from west Africa (Nicklès, 1950: 224). NHMUK 20120186, two syntypes (65.2 mm x 50.2 mm; 72.0 mm x 57.2 mm); Leeds Museum, possible syntype 1957.173.1861 (60.8 mm x 50.1 mm).

lucerna, Tellina – Hanley, 1844f: 147; 1846a: 300–301, 331, pl. 58, fig. 98. Negros & Misamis; low water, sandy mud; & Panay, hard sand [all Philippine Islands]; H. Cuming. The western Pacific *Macoma lucerna* (Hanley, 1844) (Wang, 2004b: 283, pl. 155, fig. B). NHMUK 20120187, two syntypes (figured specimen 34.4 mm x 22.4 mm; 31.9 mm x 20.9 mm).

lucinoides, *Tellina – Hanley, 1846a: 251, 330, pl. 56, fig. 32. Chain Island, South Seas. Listed on on p. 251 as “Hanley, Zool. Proc. 1844”, but not in Hanley (1844–1845). A valid species of *Abranda* (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 20120172, two syntypes (figured syntype, 12.4 mm x 10.9 mm; smaller syntype partially broken).

lux, *Tellina – Hanley, 1844f: 140; 1846a: 288–289, 331, pl. 57, fig. 71. Philippine Islands; H. Cuming. *Tellina (Angulus) lux* Hanley, 1844 (Lyngé, 1909: 201). A valid species of *Nitidotellina* (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 1907.10.28.39-40, two syntypes (figured syntype 18.7 mm x 11.3 mm; other syntype 11.5 mm x 7.7 mm) (Oliver, 1992: pl. 35, fig. 8).

lyra, *Tellina – Hanley, 1844d: 68–69; 1846a: 271–272, 332, pl. 62, fig. 187. Tumbes [Tumbes], Peru; H. Cuming. The tropical eastern Pacific *Tellina (Lyratellina) lyra* Hanley, 1844 (Coan & Valentich-Scott, 2012: 653–654), type species of *Lyratellina* Olsson, 1961, by original designation. NHMUK 20120188, two syntypes (two left valves, figured syntype 43.4 mm x 30.0 mm; other syntype 56.6 mm x 39.1 mm).

mars, *Tellina – Hanley, 1846a: 273, 332, pl. 62, fig. 180. Guinea; Cuming coll. The west African *Tellina (Peronaea) mars* Hanley, 1846 (Nicklès, 1950: 224–225). NHMUK 20120189, holotype (51.7 mm x 25.6 mm) (Salisbury, 1934: pl. 9, fig. 3). Since Hanley stated it to be “the only specimen I have ever seen,” this specimen is the holotype.

- ****micans*, *Tellina*** – Hanley, 1844d: 72; 1846a: 309, 331, pl. 59, fig. 106. Catbalonga, Samar & Bias, Negros [all Philippine Islands]; H. Cuming. The Indo-Pacific *Pulvinus micans* (Hanley, 1844) (Lynge, 1909: 204–205; Scarlato, 1965: 92, pl. 12, fig. 7; Hylleberg & Kilburn, 2003: 201; Wang, 2004b: 284, pl. 155, fig. C). *Tellina micans* Hanley, 1844, is the type species of *Pulvinus* Scarlato, 1965, by original designation. NHMUK 1976132/1, figured syntype (2 valves, 1 broken prior to 1984); NHMUK 1976133/1–3, three syntypes.
- ****miles*, *Tellina*** – Hanley, 1844f: 146–147; 1846a: 309, 332, pl. 62, fig. 188. Loc. unknown; Metcalfe coll. Type material not found in NHMUK or Leeds Museum in 2012.
- ****nobilis*, *Tellina*** – Hanley, 1845a: 165; 1846a: 300, 331, pl. 60, fig. 143. Orion, Bataan, Luzon [Philippine Islands]; low water, fine black sand; H. Cuming. *Macoma nobilis* (Hanley, 1845) from China and the Philippine Islands (Wang, 2004b: 283, pl. 155, fig. E, as “1844”). NHMUK 20120190/1, lectotype (selected by Salisbury, 1934: 81, pl. 9, fig. 1, as “holotype”) (36.1 mm x 22.8 mm); NHMUK 20120191/2, paralectotype (35.7 mm x 22.1 mm).
- ****nux*, *Tellina*** – Hanley, 1844d: 62–63; 1846a: 251–252, 330, pl. 56, fig. 33. St. Nicolas, Zebu [Philippine Islands]; 4 fms.; sandy mud; H. Cuming. *Pinguitellina nux* (Hanley, 1844) from the Philippine Islands (M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.
- ****owenii*, *Tellina*** – Hanley, 1845a: 164; 1846a: 235, 331, pl. 60, fig. 133. Africa; Zoological Society of London & British Museum colls. The west African *Tellina* (*Serratina*) *owenii* Hanley, 1845; figured by Nicklès (1950: 221–222, fig. 428), as *Quadrans chetelati* Fischer-Piette & Nicklès, 1946, a synonym, as he later recognized (Nicklès, 1955: 213). NHMUK 1951.5.21.1–3, four syntypes (three paired valves and one loose valve; figured syntype 35.6 mm x 20.5 mm).
- ****perplexa*, *Tellina*** – Hanley, 1844f: 149; 1846a: 269, 331, pl. 60, fig. 139. Manila Bay [Philippine Islands]; 6 fms. [11 m], sandy mud; H. Cuming. The western Pacific *Serratina perplexa* Hanley, 1844 (Higo et al., 2001: 170, fig. B941; Poppe & Langleit, 2011: pl. 1160, fig. 7, as *Tellina*; M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1996477, two possible syntypes (39.3 mm x 29.0 mm; 34.4 mm x 26.8 mm) (Higo et al., 2001: 170, fig. B941); Leeds Museum 1957.173.1862, one possible syntype (31.3 mm x 21.2 mm).
- ****pharaonis*, *Tellina*** – Hanley, 1844f: 148; 1846a: 235, 332, pl. 63, fig. 215. Red Sea; Metcalfe coll. Type species (subsequent designation Salisbury, 1934) of *Pharaonella* Lamy, 1918. A valid Red Sea species (M. Huber, pers. comm., 20 Dec. 2011), as recognized by Afshar (1969: 37, pl. 10, figs. 1–4), and not a synonym of *Tellina* (*Pharaonella*) *perna* Spengler, 1798, as suggested by Oliver (1992: 153). Type material not found in NHMUK or Leeds Museum in 2012.
- ****philippinarum*, *Tellina*** – Hanley, 1844d: 69; 1844g: pl. 14, fig. 18; 1846a: 279, 330, pl. 57, figs. 55, 56; 1846c: 9 (pl. expl.); 1856a: 347. San Nicolas, Zebu, & Jimmamailan, Negros [Philippine Islands]; H. Cuming. The western Pacific *Moerella philippinarum* Hanley, 1844 (Hylleberg & Kilburn, 2003: 200; Wang, 2004b: 285, pl. 156, fig. E); *Tellina*, s.l. (M. Huber, pers. comm. 2 Sept. 2012). NHMUK 1976129, two syntypes (specimen in fig. 56 is 26.4 mm x 19.8 mm; other syntype 25.6 mm x 19.1 mm; specimen in fig. 55 cannot be located) (Higo et al., 2001: 171, fig. B975, figured syntype, 1976129/1).
- ****pinguis*, *Tellina*** – Hanley, 1844d: 63; 1846a: 252, 330, pl. 56, fig. 34. St. Nicolas, Zebu [Philippine Islands]; H. Cuming. The Indo-Pacific *Pinguitellina pinguis* Hanley, 1844 (Lynge, 1909: 191–192; Oliver, 1992: 151–152, pl. 30, fig. 3a, b; Hylleberg & Kilburn, 2003: 200; Wang, 2004b: 287, pl. 157, fig. D; M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1874.12.11.340, possible syntype (figured by G. B. Sowerby II, 1867: fig. 172; Higo et al., 2001: 170, fig. B959).
- ****plebeia*, *Tellina*** – Hanley, 1844f: 147; 1846a: 299, 331, pl. 59, fig. 129, 332, pl. 60, fig. 151. “Real Llejos, Central America” [Corinto, Nicaragua]; 7 fms. [13 m], sandy mud; H. Cuming”. The specimen actually proved to be from West Africa; indeed, Hanley (1846a: 299), added “Senegal” as a locality. A synonym of *Tellina cumana* O. G. Costa, 1829 (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 1966536, two syntypes (50.9 mm x 35.5 mm, possibly that figured in fig. 151; 41.8 mm x 28.2 mm, fig. 129); Leeds Museum 1957.173.24643, possible syntype (37.1 mm x 24.9 mm).
- ****plectrum*, *Tellina*** – Hanley, 1846a: 265–266, 332, pl. 61, fig. 177. Loc. not stated; Gray coll. Listed on p. 265 as “Hanley, Zool. Proc. 1844”, but not included in that paper (Hanley, 1844–1845). Status not yet resolved. NHMUK 20120191, holotype (51.4 mm x 40.2 mm, ex

Gray collection). Hanley's original description stated that: "A single valve in the collection of J. E. Gray, Esq. is all I have ever seen of this distinctly characterized species."

princeps, *Tellina – Hanley, 1844d: 62; 1846a: 238–239, 332, pl. 63, fig. 206. Tumbes [Tumbes], Piura, Peru; 5 fms. [9 m], soft sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Tellinidella*) *princeps* Hanley, 1844 (Coan & Valentich-Scott, 2012: 657–658). NHMUK 20120191, lectotype (selected by Salisbury, 1934: pl. 9, fig. 2, as "holotype" per ICZN Code Article 74.6) (74.8 mm x 41.8 mm) (additional syntypes not located in 2012).

prora, *Tellina – Hanley, 1844d: 61; 1846a: 243, 332, pl. 60, fig. 152. Santa Elena, Ecuador; 6 fms. [11 m]; sandy mud, & Salango, Ecuador; 9 fms. [16 m], sandy mud; both H. Cuming. The tropical eastern Pacific *Tellina* (*Eurytellina*) *prora* Hanley, 1844 (Coan & Valentich-Scott, 2012: 637–638). NHMUK 1852.5.26.59, syntype (41.2 mm x 25.5 mm).

pudica, *Tellina – Hanley, 1844d: 62; 1846a: 246–247, 330, pl. 56, fig. 7. Catbalonga, Samar [Philippines]; 10 fms. [18 m], soft mud; H. Cuming. The western Pacific *Tellina* (*Arcopagia*) *pudica* Hanley, 1844 (Lynge, 1909: 194, pl. 3, figs. 35–37). Type material not found in NHMUK or Leeds Museum in 2012.

puella, Tellina – Hanley, 1845a: 165. Senegal; Cuming & Metcalfe colls. Later, Hanley (1846a: 255), correctly synonymized this species with *Tellina ampullacea* Philippi, 1844, from Senegal. Type material not found in NHMUK or Leeds Museum in 2012.

pumila, Tellina – Hanley, 1844d: 69; 1846a: 279, 330, pl. 57, fig. 41. Valparaíso [Chile]; 7–30 fms., sandy mud; H. Cuming. A mislocalized Chinese *Tellina*, s.l. (M. Huber, pers. comm., 2 Sept. 2012). NHMUK 20120193/1, lectotype (selected by Salisbury, 1934: pl. 13, fig. 3, as "holotype") (22.6 mm x 16.6 mm); NHMUK 20120193/2, paralectotype (23.3 mm x 17.3 mm; is the specimen figured by Hanley on fig. 41).

rastellum, *Tellina – Hanley, 1844d: 59 – Sept.; 1844g: pl. 14, fig. 14; 1846a: 225, pl. 64, fig. 231, pl. 65, fig. 242; 1846c: 9 (pl. expl.); 1856a: 347. Zanzibar; Cuming & Stainforth colls. The Indo-Pacific *Tellina* (*Tellinella*) *rastellum* Hanley, 1844 (Oliver, 1992: 148, pl. 33, fig. 5a, b). However, this is a junior synonym of *Tellinella philippii* (Philippi, 1844, ex Anton ms – June), described earlier in the same year (Kilburn, 1974: 347). NHMUK 1976122, two syntypes (Cuming coll.) (77.3

mm x 36.1 mm; 82.2 mm x 37.4 mm) (both syntypes are smaller than the measurements given by Hanley (3.6 inches by 1.6 inches, c. 90 mm x 40 mm), which may correspond to the specimen from the Stainforth collection, not located).

regia, *Tellina – Hanley, 1844d: 61; 1846a: 240–241, 331, pl. 60, fig. 140. "Real Llejos, Central America" [Corinto, Nicaragua]; 7 fms. [13 m], coarse sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Eurytellina*) *regia* Hanley, 1844 (Coan & Valentich-Scott, 2012: 637–639). NHMUK 1966496, two syntypes (41.9 mm x 25.7 mm; 37.6 mm x 21.1 mm).

rhodon, *Tellina – Hanley, 1844f: 140 (as "*rodon*"); 1846a: 297, 331, pl. 58, fig. 93. Loc. unknown; Cuming coll. Hanley's 1844 description of "*rodon*" noted its rose coloration, and in 1846 he silently corrected the spelling to "*rhodon*," Greek for rose, but did not explain that he was making this correction. Under the ICZN Code, this is an unjustified emendation that has gained acceptance under ICZN Article 33.3.1 ("when an incorrect subsequent spelling is in prevailing usage and is attributed to the publication of the original spelling, the subsequent spelling and attribution are to be preserved and the spelling is deemed to be a correct original spelling."). Here, *Tellina rhodon* has been frequently cited in the literature, thereby satisfying Article 33.3.1 – it was reported from Java by Prashad (1932a: 194, pl. 5, figs. 37–38), who listed a number of earlier citations) and from the Philippine Islands by Faustino (1928: 89). NHMUK 20120194, figured syntype (20.6 mm x 11.1 mm).

robusta, *Tellina – Hanley, 1844d: 63; 1846a: 252–253, 330, pl. 56, figs. 2, 3. Isle of Annaa, "South Seas" [Anaa Island, Tuamotus] & Burias [Philippine Islands]; low water, sandy mud; H. Cuming. Type species by original designation of *Pinguitellina* Iredale, 1927. NHMUK 1996482, possible syntypes (one intact specimen and nine loose valves; label gives locality as "Tahiti") (Higo et al., 2001: 170, fig. 958). As E. A. Smith (1885: 271) noted, Cuming's collection included many specimens from "Annaa Island," which is east of Tahiti, and now known as Anaa Island (Tuamotus).

rodon, Tellina; see above: *rhodon, Tellina*

rubescens, *Tellina – Hanley, 1844d: 60; 1846a: 242–243, 332, pl. 60, fig. 153. Panama and Tumbes [Tumbes], Peru; sandy mud; H. Cuming. The tropical eastern Pacific *Tellina* (*Eurytellina*) *rubescens* Hanley, 1844 (Coan

& Valentich-Scott, 2012: 639–640). NHMUK 20120195, two syntypes (figured syntype 32.0 mm x 19.8 mm; other syntype 43.5 mm x 27.3 mm).

scalpellum, *Tellina – Hanley, 1844f: 147; 1846a: 310, 331, pl. 59, fig. 116. Zebu [Philippine Islands]; low water, sandy mud; H. Cuming. Valid species of *Tellina*, s.l. (M. Huber, 20 Dec. 2011). NHMUK 1976124, three syntypes.

semen, *Tellina – Hanley, 1845a: 164; 1846a: 249–250, 330, pl. 56, fig. 8. Corregidor [Philippine Islands]; 12 fms. [22 m], sandy mud; H. Cuming. The western Pacific *Cadella semen* (Hanley, 1845) (Lynge, 1909: 198–199; Wang, 2004b: 287, pl. 157, fig. C, as “1844”). NHMUK 20060797, two possible syntypes (four valves), but from Torres Strait (Australia); largest figured by Simone & Wilkinson (2008: 152, figs. 39–34).

senegalensis, *Tellina* – Hanley, 1844d: 68; 1846a: 259–260, pl. 56, fig. 17. Senegal. *Non Tellina senegalensis* Gmelin, 1791. Synonym of the west African *Strigilla* (*Aeretica*) *splendida* (Anton, 1838) (E. A. Smith, 1915; Salisbury, 1934: 89). E. A. Smith (1915) proposed a “nom. nov.”, *Strigilla tomlini*, for the *S. splendida* (Anton) figured by Hanley (1846: 259, pl. 56, fig. 39) from the Philippine Islands, but this has to be interpreted as a new species, not a new name. Thus, the “lectotype” of Hanley’s species (NHMUK 1996480) later figured by Salisbury (1934: pl. 11, fig. 1), is, in fact, the holotype of *S. tomlini* E. A. Smith, 1915. *Tellina senegalensis* Hanley, 1844, is the type species of *Aeretica* Dall, 1900, by original designation.

sincera, *Tellina {XE “**sincera, *Tellina***”} – Hanley, 1844d: 68; 1846a: 261, 331, pl. 60 [“59” in error on p. 261], fig. 144. Loc. unknown; Cuming & Metcalfe colls. Australian authors have treated this as a valid Australian species, *Strigilla* (*Strigilla*) *sincera* (Hanley, 1844) (Hedley, 1913: 272–273; Lamprell & Whitehead, 1992: pl. 48, fig. 356). Hedley (1913: 273) stated that he “found two unnamed *Strigilla* labeled ‘Moreton Bay, Mr. Strange,’” which E. A. Smith and he “agreed to identify [as] the type of Hanley’s *Strigilla sincera*.” Hedley (1913: 273) concluded that records from Panama (eastern Pacific) of this species were misidentified specimens of *Strigilla disjuncta* Carpenter, 1856. However, M. Huber (pers. comm. 2 Sept. 2012) determined that the type material did, in fact, correspond to the eastern Pacific species of

Strigilla, and the Australian species is instead *Strigilla* (*Strigilla*) *grossiana* Hedley, 1908, which is distinct from Hanley’s type material identified by Hedley. Hence, Hanley’s species would be a senior synonym of *Strigilla disjuncta* Carpenter, 1856 (for the latter, see Coan & Valentich-Scott, 2012: 621–622). NHMUK 20120171, two syntypes (30.6 mm x 27.6 mm; 29.8 mm x 26.3 mm).

sol, *Tellina* – Hanley, 1844f: 142–143; 1846a: 275, 333, pl. 65, fig. 240. Loc. unknown [“restricted” to St. Croix, Virgin Islands (Boss, 1966)]; Cuming & Metcalfe colls. Synonym of the western Atlantic *Laciolina magna* (Spengler, 1798) (Boss, 1966: 249–251, pl. 133, figs. 4, 5; pl. 134, fig. 4, as *Tellina*). NHMUK 1987059, two syntypes (104.7 mm x 60.0 mm; 95.2 mm x 55.9 mm). Boss (1966: 249–250) stated that the smaller specimen was the “holotype,” but this was not a valid lectotype selection under ICZN Code Article 74.5 as Hanley referred to specimens from the Cuming and the Metcalfe collections.

souleyeti, *Tellina – Hanley, 1844d: 71–72; 1846a: 308–309, 331, pl. 59, fig. 111. San Nicolas, Zebu [Philippine Islands]; low water, sandy mud; H. Cuming. Type material not found in NHMUK or Leeds Museum in 2012.

sowerbii, *Tellina – Hanley, 1844d: 62; 1846a: 247, 332, pl. 63, fig. 205. Loc. unknown; Sowerby & Hanley colls. The Australian *Laciolina sowerbii* (Hanley, 1844) (Lamprell & Whitehead, 1992: pl. 43, fig. 301, as *Tellina*). NHMUK 1976131/1, figured syntype (68.5 mm x 39.0 mm); NHMUK 1976131/2, syntype (one valve, 68.0 mm x 39.1 mm); Leeds Museum 1957.173.1860, syntype (59.2 mm x 33.3 mm).

spectabilis, *Tellina – Hanley, 1844f: 141; 1846a: 323, 333, pl. 65, fig. 254. Manila Bay & Siquijor [Philippine Islands], low water, coral sand; H. Cuming. The southeast Asian and Philippine Islands *Leporimetis spectabilis* (Hanley, 1844) (Scarlato, 1965: 75, pl. 7, fig. 4; Hylleberg & Kilburn, 2003: 197; Wang, 2004b: 282, pl. 155, fig. J). The material reported as this species from Australia (Lamprell & Whitehead, 1992: pl. 50, fig. 37) is instead a distinct species (M. Huber, pers. comm., 2 Sept. 2012). *Tellina spectabilis* Hanley, 1844, is the type species of *Leporimetis* Iredale, 1930, by original designation. NHMUK 199484, two possible syntypes (63.0 mm x 55.4 mm; 63.7 mm x 59.5 mm) (Higo et al., 2001: 171, fig. B1006).

- ***spinosa, *Tellina*** – Hanley, 1844f: 148–149; 1846a: 264–265, pl. 57 (as “58” on p. 264), fig. 40. Ticao [Philippine Islands]; H. Cuming. The Philippine Islands *Quadrans spinosus* (Hanley, 1844) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1986093, holotype (19.0 mm x 14.1 mm) (Hanley referred to “Mr. Cuming’s unique specimen,” so this is a holotype) (Higo et al., 2001: 170, fig. B957).
- ***subrosea, *Tellina*** – Hanley, 1846a: 298, 330, pl. 57, fig. 64. Catbalonga, Samar [Philippine Islands]; soft mud; H. Cuming. Possibly referable to *Nitidotellina*, but lacking type material and not definitively identifiable, so better treated as a *nomen dubium* (M. Huber, pers. comm., 2 Sept. 2012). Type material not found in NHMUK or Leeds Museum in 2012.
- ***subtruncata, *Tellina*** – Hanley, 1844f: 149; 1846a: 367, pl. 57, fig. 48. Bohol [Philippine Islands]; low water, on reefs; H. Cuming. *Pristipagia subtruncata* (Hanley, 1844), from the Philippine Islands (Salisbury, 1934: 85, pl. 11, fig. 2, as *Arcopagia*; M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1976121, two syntype valves (17.3 mm x 13.8 mm) (Higo et al., 2001: 170, fig. B944). Salisbury (1934: 85) referred to these valves as the “holotype,” but Hanley referred to more than one specimen (Cuming and Belcher collections, the latter not located in 2012), so this was not a valid lectotype selection (ICZN Code Article 74.5).
- ***tulipa, *Tellina*** – Hanley, 1844f: 148; 1846a: 232–233, 330, pl. 56, fig. 25. Loc. unknown; Cuming & Walton colls. NHMUK 20120196, figured syntype (21.1 mm x 12.1 mm). Salisbury (1934: pl. 9, fig. 2) stated that this specimen was the “holotype,” but Hanley referred to more than one specimen (Cuming collection and Walton collection, the latter not located in 2012), so this was not a valid lectotype selection (ICZN Code Article 74.5).
- ***undulata, *Tellina*** – Hanley, 1844d: 72; 1846a: 310, 331, pl. 59, fig. 107. Santa Elena, West Colombia [Guayas, Ecuador]; 6 fms. [11 m], sandy mud; H. Cuming. The tropical eastern Pacific *Cymatoica undulata* (Hanley, 1844) (Coan & Valentich-Scott, 2012: 601–602). *Tellina undulata* Hanley, 1844, is the type species of *Cymatoica* Dall, 1900, by original designation. NHMUK 1874.12.11.329, syntype (16.7 mm x 9.3 mm).
- ***valtonis, *Tellina*** – Hanley, 1844f: 143; 1846a: 283–284, 330, pl. 57, fig. 68. Loc. unknown; Metcalfe coll. The Japanese *Nitidotellina valtonis* (Hanley, 1844). The figures in Oliver (1992), Lamprell & Whitehead (1992: pl. 44, fig. 308, as *Tellina*) and Poppe & Langleit (2011) are misidentified specimens of other species (M. Huber, pers. comm., 2 Sept. 2012).
- ***vernalis, *Tellina*** – Hanley, 1844f: 141; 1846a: 289, 331, pl. 58, fig. 84. Singapore, 7 fms. [13 m], sandy mud; H. Cuming. Probably referable to *Nitidotellina* (M. Huber, pers. comm. 2 Sept. 2012). The illustrations in Lamprell & Whitehead (1992: pl. 44, fig. 310) and Oliver (1992: pl. 35, fig. 8) refer to other species (M. Huber, pers. comm., 2 Sept. 2012). Type material not found in NHMUK or Leeds Museum in 2012.
- ***verrucosa, *Tellina*** – Hanley, 1844d: 60; 1846a: 225, pl. 68, fig. 77. Corregidor, Manila [Philippine Islands]; H. Cuming. *Scutarcopagia verrucosa* (Hanley, 1844) from the Philippine Islands (Higo et al., 2001: 169, fig. B917, syntype; Poppe & Langleit, 2011: pl. 1154, figs. 2, 3, as *Tellina*; M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1976125, syntypes (figured syntype 35.4 mm x 17.4 mm) (Higo et al., 2001: 169, fig. B917). Hanley specified that: “Two specimens ... only are as yet known,” but NHMUK 1976125 contains three specimens, so one must have been added at a later date.
- ***vestalis, *Tellina*** – Hanley, 1844f: 141; 1846a: 291, 330, pl. 57, fig. 67 (as “[fig.] 97” on p. 330). Negros, 7 fms. [13 m], coral sand, & Luzon, 6 fms. [11 m], mud [Philippine Islands]; H. Cuming. The southeast Asian *Tellina vestalis* Hanley, 1844 (Lynge, 1909: 198; Hylleberg & Kilburn, 2003: 196; Wang, 2004b: 279, pl. 154, fig. F; Poppe & Langleit, 2011: pl. 1156, fig. 3 only, not fig. 4). NHMUK 1993044, three syntypes (figured syntype 28.8 mm x 15.3 mm; other two syntypes are smaller).
- ***virgo, *Tellina*** – Hanley, 1844f: 143; 1846a: 284–285, 330, pl. 57, fig. 42. Loc. unknown; H. Cuming. The tropical eastern Pacific *Tellina* (*Scissula*) *virgo* Hanley, 1844 (Coan & Valentich-Scott, 2012: 656, 658). NHMUK 20120197/1, lectotype (23.1 mm x 13.8 mm) (selected by Salisbury, 1934: pl. 13, fig. 5, as “holotype” pursuant to ICZN Code Article 74.6); NHMUK 20120197/2, paralectotype (19.4 mm x 11.1 mm) (Salisbury, 1934: pl. 13, fig. 6).
- ***virgulata, *Tellina*** – Hanley, 1845a: 164; 1846a: 231, 330, pl. 56, fig. 5. Loc. unknown; Cuming coll. NHMUK 20120198, possible syntype (15.0 mm x 7.8 mm) (same size as fig. 5, and slightly shorter than measurements given by Hanley, 0.3 inches by 0.7 inches).

vulsella, Tellina – Hanley, 1846a: 235–236, 332, pl. 61, figs. 162, 163, ex Chemnitz ms. Zebu [Cebu], Philippine Islands. Synonym of the western Pacific *Pharaonella aurea* (Perry, 1811) (Petit, 2003: 27; Poppe & Langleit, 2011: pl. 1155, fig. 2). Leeds Museum 1957.173.174, one specimen, with note in collection by S. Whybrow (Morris), “unlikely to be a type” (Sept. 1979) (64.0 mm x 25.4 mm).

Donacidae

Note: in his 1843 publications, Hanley treated the genus *Donax* as having feminine gender, with the species names ending in “a”, whereas in his 1845 and subsequent publications, he correctly treated this genus as having masculine gender, with the species names usually ending in “s”.

***asper, Donax** – Hanley, 1845d: 14–15. Tumbes [Tumbes], Peru; H. Cuming. The tropical eastern Pacific *Donax* (*Assimilidonax*) *asper* Hanley, 1845 (Coan & Valentich-Scott, 2012: 675–676). NHMUK 198212/1, lectotype (selected by Coan, 1983: 283, fig. 22); NHMUK 198212/2–3, two paralectotypes; NHMUK 198213, three probable paralectotypes.

***assimilis, Donax** – Hanley, 1845e: 17. Panama; Cuming & Hanley colls.; probably collected by H. Cuming. The tropical eastern Pacific *Donax* (*Assimilidonax*) *assimilis* Hanley, 1845 (Coan & Valentich-Scott, 2012: 675–676). *Donax assimilis* Hanley 1843, is the type species of *Assimilidonax* Huber, 2010, by original designation. NHMUK 198214/1, lectotype (selected by Coan, 1983: 282, fig. 17); NHMUK 198214/2–3, two paralectotypes.

***carinata, Donax** – Hanley, 1843b: 84; 1843c: 5–6; 1844g: pl. 14, fig. 28; 1846c: 9 (pl. expl.). Mus. Stainforth & Metcalfe collection (1843c). Loc. not stated. The tropical eastern Pacific *Donax* (*Carinadonax*) *carinatus* Hanley, 1843 (Coan & Valentich-Scott, 2012: 671–673). *Donax carinatus* Hanley, 1843, is the type species of *Carinadonax* Huber, 2010; by original designation. NHMUK 1900.2.8.4, syntype (figured by Coan, 1983: 285, fig. 24, as “presumably the holotype,” but the species was described from specimens in two collections).

***culter, Donax** – Hanley, 1845d: 14. Mazatlan & Acapulco, Mexico; Cuming coll. The tropical eastern Pacific *Donax* (*Chion*) *culter* Hanley, 1845 (Coan & Valentich-Scott, 2012: 667–668). NHMUK 1966547/1, lectotype (selected by Coan, 1983: 280, fig. 12); NHMUK 1966547/2–3, two paralectotypes.

***dentifera, Donax** – Hanley, 1843b: 84; 1843c: 6; 1844g: pl. 14, fig. 20; 1846c: 9 (pl. expl). “S. America?” [tropical eastern Pacific]. The tropical eastern Pacific *Donax* (*Assimilidonax*) *dentifer* Hanley, 1843 (Coan & Valentich-Scott, 2012: 674–675). NHMUK 1912.6.18.28, lectotype (selected by Coan, 1983: 283, fig. 20); paralectotype(s) not located in NHMUK or Leeds Museum in 2012.

flavidus, Donax – Hanley, 1882a: 540–541, pl. 12, fig. 3. Borneo; ex Geale coll. Synonym of the southeast Asian *Donax* (*Dentilatona*) *incarnatus* Gmelin, 1791 (Huber, 2010: 319, 690). NHMUK 1900.3.9.24, holotype (9.6 mm x 11.4 mm). Hanley referred to “this very rare shell, of which I have never seen but one example,” so this is the holotype.

***gracilis, Donax** – Hanley, 1845d: 15. Golfo de Guayaquil & Bahía de Caráquez, Ecuador; & Chiriqui, Panama; H. Cuming. The tropical eastern Pacific *Donax* (*Paradonax*) *gracilis* Hanley, 1845 (Coan & Valentich-Scott, 2012: 673–674). NHMUK 1966551/1, lectotype (selected by Coan, 1983: 290, fig. 320); NHMUK 1966551/2–3, two paralectotypes. NHMUK 198221 has two specimens that correspond to “var. c” of this species.

***impar, Donax** – Hanley, 1882a: 541, pl. 12, fig. 4. Beloochistan [Balochistan, Pakistan]; Blanford. *Donax* (*Machaerodonax*) *impar* Hanley, 1882, from the Indian Ocean (Huber, 2010: 320). NHMUK 1904.10.28.138, holotype (one valve, 14.8 mm x 5.7 mm). Hanley stated: “I possess only a single valve”, so this is the holotype.

***listeri, Donax** – Hanley, 1882a: 540, pl. 12, fig. 2. Loc. unknown; ex Belcher coll. The Southeast Asian *Donax* (*Latona*) *listeri* Hanley, 1882 (Huber, 2010: 381). NHMUK 1900.3.9.25, holotype (32.9 mm x 26.9 mm). Hanley referred to “our unique specimen”, so this is the holotype.

***lubricus, Donax** – Hanley, 1845e: 17. Loc. unknown; Cuming coll. The South African *Donax* (*Dentilatona*) *lubricus* Hanley, 1845 (Huber, 2010: 319). NHMUK 1980099, syntype (figured by Reeve, 1854: pl. 7, fig. 46); NHMUK 1980100, syntype.

mesodesmoides, Donax – Hanley, 1882a: 539–540, pl. 12, fig. 1. Loc. unknown. Synonym of the South African *Donax* (*Donax*) *serra* Röding, 1798 (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1907.10.28.109, holotype (43.9 mm x 27.0 mm). Hanley referred to “This unique and aberrant species,” and the rest of the description implies that it was based on a single specimen.

navicula, *Donax* – Hanley, 1845d: 15. Golfo de Nicoya, Central America [Costa Rica]; H. Cuming. Synonym of the eastern Pacific *Donax* (*Paradonax*) *californicus* Conrad, 1837 (Coan & Valentich-Scott, 2012: 673–674). NHMUK 1967697/1, lectotype (selected by Coan, 1973: 134; figured in Coan, 1983: 289, fig. 31); NHMUK 1967697/2–3, two paralectotypes (in 1981, S. Morris found the missing left valve of the first paralectotype, and a third specimen, which she stated in a note in the collection was “presumed to be another syntype”).

****owenii, Donax*** – Hanley, 1843b: 81–82, ex Gray ms. Africa. The West African *Donax* (*Tentidonax*) *owenii* Hanley, 1843 (Nicklès, 1950: 213; Huber, 2010: 317). NHMUK 1981248, five syntypes.

****pulchella, Donax*** – Hanley, 1843b: 86; 1843c: 6; 1844g: pl. 14, fig. 19; 1846c: 9 (pl. expl.). “West Indies?” The West African *Donax* (*Donax*) *pulchellus* Hanley, 1843 (Nicklès, 1950: 213; Huber, 2010: 313). NHMUK 1900.2.8.44, figured syntype (single left valve); NHMUK 1900.2.8.21, one syntype; NHMUK 1900.2.8.22–25, four syntypes.

****punctatostriata, Donax*** – Hanley, 1843b: 84–85; 1843c: 5, both as “*punctato-striata*”; 1844g: pl. 14, fig. 24; 1846c: 9 (pl. expl.), as “*punctato-striatus*”. “China” [actually tropical eastern Pacific]. The tropical eastern Pacific *Donax* (*Chion*) *punctatostriatus* Hanley, 1843 (Coan & Valentich-Scott, 2012: 670–671). NHMUK 1912.6.18.27, lectotype (selected by Coan, 1983: 275, fig. 1); paralectotype(s) not located in NHMUK or Leeds Museum in 2012.

****semisulcata, Donax*** – Hanley, 1843b: 85; 1843c: 5; 1844g: pl. 14, fig. 25; 1846c: 9 (pl. expl.). Loc. not stated. The Southeast Asian *Donax* (*Deltachion*) *semisulcatus* Hanley, 1843 (Hylleberg & Kilburn, 2002: 67; Huber, 2010: 321). NHMUK 1907.10.28.143–144, two syntypes (“E. Africa”).

****sordidus, Donax*** – Hanley, 1845d: 15–16. Cape of Good Hope, South Africa; Cuming & British Museum colls. The South African *Donax* (*Assimilidonax*) *sordidus* Hanley, 1856 (Huber, 2010: 315). NHMUK 1840.10.23.165, one syntype; NHMUK 2012069, one syntype.

****ticaonicus, Donax*** – Hanley, 1845d: 14. Ticao [Philippine Islands]; H. Cuming. The Indo-Pacific *Donax* (*Latona*) *ticaonicus* Hanley, 1845 (Huber, 2010: 318). NHMUK 1996449, possible syntype (Higo et al., 2001: 174, fig. B1081).

Psammobiidae

costata, Psammobia – Hanley, 1843a: 122–123; 1843b: 59; 1843f: 5 (pl. expl.); 1843g: pl. 11, fig. 12. New Zealand (1843a); Australia (1843b). Hanley’s paper (1843a) was misattributed to Reeve by both Sherborn (1925: 1576) and Willan (1993: 49, 100), whereas Reeve merely conveyed the paper by Hanley to the Zoological Society, as he did for some others in 1842–1843, before Hanley was on hand to present his own work. Willan (1993: 49) concluded that Hanley’s species was based on mislocalized material of the North Atlantic *Gari virgata* (Lamarck, 1818). Type material not found in NHMUK or Leeds Museum in 2012.

Semelidae

****carnicolor, Amphidesma*** – Hanley, 1845b: 162; 1843f: 6 (pl. expl.); 1843g: pl. 12, fig. 28; 1856a: 342. Philippine Islands; Cuming & Hanley colls.; possibly collected by H. Cuming. The Indo-Pacific *Semele carnicolor* (Hanley, 1845) (Oliver, 1992: 167, pl. 30, fig. 5a, b; Hylleberg & Kilburn, 2003: 203; Huber, 2010: 338). NHMUK 1907.10.28.137, possible syntype (24.9 mm x 23.9 mm) (Higo et al., 2001: 172, fig. B1009); NHMUK 20110072, three syntypes (including specimen later figured by Reeve, 1853: pl. 1, fig. 6).

****gravida, Leptomya*** – Hanley, 1879: 580–581; 1882a: 541, pl. 12, fig. 5. Loc. unknown; Hanley coll. Possibly a distinct, poorly known species (Huber, 2010: 702). NHMUK 1907.10.28.99, holotype (one left valve, from “Aracan” [Burma]) (30.1 mm x 23.2 mm). Hanley stated “I have only seen a single valve (a left one)” of this species, so this specimen is the holotype.

multicostatum, Amphidesma – G. B. Sowerby I, 1841: 8, *nomen nudum*; Hanley, 1858f: *Amphidesma* pl. 1, fig. 11, ex G. B. Sowerby I ms. Status unresolved. Type material not found in NHMUK or Leeds Museum in 2012.

****psittacus, Leptomya*** – Hanley, 1882a: 541, pl. 12, fig. 6; 1882b: 576. Loc. unknown; Hanley coll. The Indian Ocean *Leptomya psittacus* Hanley, 1882 (Hylleberg & Kilburn, 2003: 203; Huber, 2010: 343). NHMUK 1907.10.28.100, syntype (30.8 mm x 22.2 mm). Although Hanley referred to “this very rare shell,” he did not state that it was the unique specimen, so the NHMUK specimen is not a holotype.

scabrum, *Amphidesma – Hanley, 1843f: 6 (pl. expl.); 1843g: pl. 12, fig. 9; 1844c: 17; 1856a: 342 (pl. 13 mistakenly cited). Boljoon, Zebu. Attributed to W. Metcalfe in Sherborn (1930: 5765). The Southeast Asian *Semele scabra* (Hanley, 1843) (Wang, 2004b: 294, pl. 160, fig. H; Huber, 2010: 339; Poppe, 2011b: pl. 1177, figs. 9, 10). Hanley gave the measurements of this species as 0.2 inches by 2.5 inches, an obvious error. Type material not found in NHMUK or Leeds Museum in 2012. Solene Whybrow (later Morris) determined that Leeds Museum 1957.173.618, which is labeled as this species, was not the type specimen, according to her note in the collection (Sept. 1979).

spectabilis, *Leptomya* – Hanley, 1882a: 541, pl. 12, fig. 7; 1882b: 576–577. “Japan?” (1882b); Hanley coll. Synonym of *Leptomya rostrata* (H. Adams, 1868) from the Red Sea (Huber, 2010: 343, 701–702). NHMUK 1907.12.30.527, syntype (27.7 mm x 20.1 mm). The original description also refers to specimens in the Taylor collection and the British Museum collection, so this NHMUK lot is not a holotype.

zebuense, *Amphidesma – Hanley, 1843f: 6 (pl. expl.); 1843g: pl. 12, fig. 7; 1844c: 17–18; 1856a: 342. Zebu, Philippine Islands. Attributed to W. Metcalfe in Sherborn (1932: 7038). This species dates from the appearance of the plate corresponding to the previously published plate explanation. *Semele zebuensis* (Hanley, 1843) from the Philippine Islands (Huber, 2010: 339). The figures in Poppe labeled as being this species (2011b: pl. 1177, figs. 5–8) are instead *Semele duplicata* (Reeve, 1853) and *Semele exarata* (A. Adams & Reeve, 1850) (M. Huber, pers. comm., 28 May 2012). NHMUK 1996452, three possible syntypes (Higo et al., 2001: 171, fig. B1007).

Solecurtidae

bidens, *Solen* – Hanley, 1842b: 16, pl. 2, fig. 24; 1843f: 2 (Syst. List; mistakenly referencing pl. 3, fig. 11), [2] (expl. to pl. 2); 1856a: 336, ex Chemnitz ms. Based in part on Chemnitz (1795: pl. 198, fig. 1939). “Nicobar Islands”. Synonym of the western Atlantic *Tagelus divisus* (Spengler, 1794) (Forbes & Hanley, 1848: 266; Dall, 1898: 62). Type material not found in NHMUK or Leeds Museum in 2012.

Mactridae

lisor, *Mactra* – “Hanley”, 1842b: 32, ex Adanson (1757); 1843b: pl. 3, fig. 57; 1843f: 4 (Syst. List), [3] (expl. to pl. 3), 6 (expl. to pl. 11); 1843g: pl. 11, figs. 54, 55; 1855: 56. Senegal. Although sometimes credited to Hanley, this pre-Linnaean Adanson name was made available still earlier by Anton, 1838. However, it is a synonym of the west African *Mactra (Mactra) glabrata* Linnaeus, 1767 (Hanley, 1855: 56; Nicklès, 1950: 208; Huber, 2010: 440, 755).

Mesodesmatidae

planum, *Mesodesma – Hanley, 1843e: 102; 1843f: 6 (pl. expl.); 1843g: pl. 12, fig. 19; 1856a: 341. Loc. unknown. Attributed to Reeve in Sherborn (1929: 5018). Type species of *Davila* Gray, 1853 [type species by monotypy: *D. polita* Gray, 1853, = *Mesodesma planum*]. *Davila plana* (Hanley, 1843) occurs in the western Indo-Pacific (Beu, 1971: 125; Matsukuma, 2000: 968–969; Qi, 2004: 274, pl. 150, fig. I). Type material not found in NHMUK or Leeds Museum in 2012.

triquetrum, *Mesodesma* – Hanley, 1843e: 101–102; 1843f: 6 (pl. expl.); 1843g: pl. 12, fig. 20; 1856a: 341. Loc. unknown. Attributed to Reeve in Sherborn (1931: 6626). Synonym of the South Australian *Anapella cycladea* (Lamarck, 1818) (Lamprell & Whitehead, 1992: pl. 41, fig. 287). Hanley's species is the type, by original designation, of *Anapella* Dall, 1895. Type material not found in NHMUK or Leeds Museum in 2012.

Ungulinidae

cumingii, *Cyrenoidea – Hanley, 1844g: pl. 15, fig. 5 (without caption); 1846c: 10 (pl. expl.); 1856a: 353. Philippine Islands. This and the next species date from when the explanation of the earlier plate is known to have appeared. Qi (2004: 257–258, pl. 142, fig. I) and Xu (2012: 71–72, fig. 40), both as “1844”, figured this as the western Pacific *Cycladicama cumingii* (Hanley, 1846). However, Huber (pers. comm., 20 Dec. 2011) considers these to have been based on misidentified specimens of *Joannisiella coreensis* (A. Adams & Reeve, 1850). The true *Cycladicama cumingii* (Hanley, 1846) occurs in Southeast Asia (Hylleberg & Kilburn, 2003: 174), and it

was included in a genetic analysis of the Heterodonta (Taylor et al., 2007: 591). NHMUK 1900.2.13.7, syntype (26.1 mm x 23.6 mm).

***oblonga, Cyrenoidea** – Hanley, 1844g: pl. 15, fig. 6 (without caption); 1846c: 10 (pl. expl.); 1856a: 353. Philippine Islands. The western Pacific *Cycladicama oblonga* (Hanley, 1846) (Hylleberg & Kilburn, 2003: 174, as “1856”; Qi, 2004: 258, pl. 142, fig. J; Xu, 2012: 69–71, fig. 39, the latter two as “1844”), or perhaps more appropriately *Joannisiella oblonga* (Hanley, 1846) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1900.2.13.8, syntype (33.4 mm x 27.3 mm).

Veneridae

***bruguieri, Venus** – Hanley, 1844g: pl. 15, fig. 59 (without caption); 1845f: 21; 1846c: 12 (pl. expl., as “brugieri”); 1856a: 362; 1859c: 237. Loc. unknown; Cuming & Hanley colls. The Indian *Venerupis* (*Ruditapes*) *bruguieri* (Hanley, 1845) (Huber, 2010: 428). Type material not found in NHMUK or Leeds Museum in 2012.

***chemnitzii, Venus** – Hanley, 1844e: 160–161; 1844g: pl. 16, fig. 20; 1846c: 13 (pl. expl.); 1856a: 360. San Nicholas, Philippine Islands; H. Cuming; Cuming & Hanley colls. The Indo-Pacific *Periglypta chemnitzii* (Hanley, 1844) (Qi, 2004: 308, pl. 167, fig. F) or *Antigona chemnitzii* (Hanley, 1844) (Poppe, 2011a: pl. 1125, figs. 1–4, as “1845”). NHMUK 196244, three syntypes (smallest figured by Hanley and by Reeve, 1863: pl. 10, fig. 32a; another syntype figured by Reeve, 1863: pl. 10, fig. 32b) (Higo et al., 2001: 175, fig. B1129, as *Periglypta*). Leeds Museum 1957.173.15189, syntype (46.2 mm x 36.6 mm); Leeds Museum 1957.173.25818, possible syntype (58.3 mm x 45.3 mm).

***cor, Cytherea** – Hanley, 1844e: 110; 1844g: 10 (pl. expl.), pl. 15, fig. 7; 1856a: 354. Africa; Metcalfe coll. The west African *Costellipitar cor* (Hanley, 1844) (Huber, 2010: 397). NHMUK 1907.10.28.16, syntype (22.0 mm x 18.1 mm).

decipiens, Venus – Hanley, 1844g: pl. 16, fig. 22; 1845b: 162; 1846c: 13 (pl. expl.); 1856a: 360. “Australia?” (1845b); Cuming & Hanley colls. By 1856a (p. 360), Hanley said that he “had reason to believe” that this species may have been based on a young, mislocalized specimen of the eastern Atlantic *Clausinella fasciata* (da Costa, 1778), and that is indeed the case (Fischer-Piette &

Vukanovich, 1977: 110–113; Huber, 2010: 715). NHMUK 1907.10.28.102, syntype (16.6 mm x 14.1 mm).

deshayesii, Venus – Hanley, 1844g: pl. 16, fig. 35; 1846c: 13 (pl. expl.); 1856a: 363. Loc. not stated (1846c); “Philippines” (1856a). Hanley’s plate explanation in 1846 included a footnote that was disclaimed in 1856. This Indo-Pacific species was listed as *Tapes deshayesi* (G. B. Sowerby II, 1852), with *Venus deshayesii* “Hanley, 1856,” in synonymy by Oliver (1992: 189–190, pl. 43, fig. 3a, b). In fact, Hanley’s species dates from the publication of Hanley’s 1846 plate explanation, but is a junior synonym of *Tapes sulcarius* (Lamarck, 1818) (M. Huber, pers. comm., 20 Dec. 2011). Leeds Museum 1957.173.25821, two possible syntypes.

***diemenensis, Cytherea** – Hanley, 1844e: 110. Van Diemen’s Land [Tasmania, Australia]; Metcalfe coll. The southern Australian *Callista* (*Striacallista*) *diemensis* (Hanley, 1844) (Lamprell & Whitehead, 1992: pl. 69, fig. 541; M. Huber, pers. comm., 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

***effossa, Cytherea** – Hanley, 1843a: 123; 1843b: 109. Loc. unknown. Attributed to Reeve in Sherborn (1926: 2080). The Indo-Pacific *Sunetta effosa* (Hanley, 1843) (Oliver, 1992: 184, pl. 42, fig. 7a, b; Hylleberg & Kilburn, 2003: 219). Leeds Museum 1957.173.24640, two possible syntypes (33.7 mm x 23.3 mm; 26.9 mm x 18.9 mm) (with note in collection by S. Whybrow (Morris), “neither of these 2 appears to be the figured specimen”) (Sept. 1979)).

excavata, Cytherea – Hanley, 1843a: 123; 1843b: 109. Loc. unknown. Attributed to Reeve in Sherborn (1926: 2262). *Non Cytherea excavata* Morton, 1833. Hanley’s species does not appear to have been renamed. Leeds Museum 1957.173.24641, syntypes (three valves, largest 21.0 mm x 16.4 mm).

hindsii, Cytherea – Hanley, 1844e: 110; 1844g: pl. 15, fig. 35; 1846c: 11 (pl. expl.); 1856a: 356. Guayaquil [Ecuador]; Cuming & Hanley colls. Synonym of the tropical eastern Pacific *Tivela* (*Tivela*) *byronensis* (Gray, 1838) (Coan & Valentich-Scott, 2012: 829–831). NHMUK 20120193, three syntypes; Leeds Museum 1957.173.24671, possible syntype (24.1 mm x 26.7 mm).

***lacerata, Venus** – Hanley, 1844g: pl. 16, fig. 23; 1845b: 161; 1846c: 13 (pl. expl.); 1856a: 360–361. Catbalonga [Philippine Islands];

Cuming & Hanley colls.; probably collected by H. Cuming. The Indo-Pacific *Periglypta lacerata* (Hanley, 1845) (Hylleberg & Kilburn, 2003: 217; Qi, 2004: 307, pl. 167, fig. C, as "1844") or *Antigona lacerata* (Hanley, 1845) (Poppe, 2011a: pl. 1127, figs. 5, 6). Type material not found in NHMUK or Leeds Museum in 2012. NHMUK 196246 consists of one specimen (figured by Reeve 1863: pl. 6, fig. 18, but from Zebu, which is not the type locality and does not match Hanley's illustration).

Lucinopsis – Forbes & Hanley, 1848: 433–438. Type species by monotypy: *Venus undata* Pennant, 1777. Junior synonym of *Mysia* Lamarck, 1818, ex Leach ms, which has the same type species by monotypy.

****lyra, Venus*** – Hanley, 1845b: 161–162; 1844g: pl. 16, fig. 21; 1846c: 13 (pl. expl.); 1856a: 360. Gulf of Guinea, Rang; Cuming & Hanley collections. The west African *Venus lyra* Hanley, 1845 (Huber, 2010: 361). NHMUK 1907.10.28.114, syntype (32.5 mm x 28.5 mm) (bottom of box states "one of TYPES from Hanley coll." although this is not the figured specimen).

****magnifica, Venus*** – Hanley, 1845f: 21–22. Ticao [Philippine Islands]; on sand; H. Cuming; Cuming coll. *Antigona magnifica* (Hanley, 1845) from the Philippine Islands (Poppe, 2011a: pl. 1128, figs. 1–4). NHMUK 196250, one syntype; NHMUK 1962177, two syntypes.

nivea, Cytherea – Hanley, 1843b: 97. Red Sea. Not listed in Oliver (1992); the white form of the Indian Ocean *Callista* (*Notocallista*) *umbo-nella* (Lamarck, 1818) (M. Huber, pers. comm., 20 Dec. 2011). NHMUK 1907.10.28.104, syntype (54.3 mm x 45.0 mm).

obliquata, Cytherea – Hanley, 1844e: 109; 1844g: pl. 15, fig. 24; 1846c: 11 (pl. expl.); 1856a: 355. Loc. unknown; Cuming & Hanley colls. The Indo-Pacific *Pitar obliquatus* (Hanley, 1844) (Qi, 2004: 299, pl. 163, fig. E). However, Lamprell & Healy (1997: 291, 293) had determined that it was a junior synonym of *Pitar* (*Pitarina*) *prora* (Conrad, 1837), based on examination of Hanley's type material. NHMUK 1912.6.4.14, syntype (49.0 mm x 44.4 mm, from "Philippines"). Hanley (1844e: 109) stated that this was in the "Mus. Cuming, Hanley, &c" making clear that there were multiple type specimens.

ovum, Cytherea – Hanley, 1844g: pl. 15, fig. 21; 1845f: 21; 1846c: 11 (pl. expl.); 1856a: 354–355. Loc. unknown; Malabar (1856a); Cuming & Hanley colls. Synonym of the

Indian Ocean *Meretrix casta* (Gmelin, 1791) (Hylleberg & Kilburn, 2003: 216; Huber, 2010: 389, 725). Leeds Museum 1957.173.756, syntype (27.7 mm x 20.3 mm); NHMUK 1912.6.18.13, possible syntype; NHMUK 1912.3.15.2–3, two possible syntypes.

****philippinarum, Cytherea*** – Hanley, 1844e: 110; 1844g: pl. 15, fig. 36; 1846c: 11 (pl. expl.); 1856a: 356. Philippine Islands; Cuming & Hanley colls. The Indo-Pacific *Lioconcha philippinarum* (Hanley, 1844) (Oliver, 1992: 186, pl. 40, fig. 4a, b; Hylleberg & Kilburn, 2003: 214). *Cytherea philippinarum* Hanley, 1844, is the type species of *Sulcilioconcha* Habe, 1951, by original designation. NHMUK 1912.6.18.20, lectotype (selected by Lamprell & Kilburn, 1999: 45, pl. 1, figs. c–e); NHMUK 1966346/1–7, six paralectotypes.

plebeia, Cytherea – Hanley, 1844e: 109–110; 1844g: pl. 15, fig. 37; 1846c: 1856a: 356. Catbalonga, Philippine Islands; Cuming & Hanley colls. Synonym of the western Indo-Pacific *Redicirce sulcata* (Gray, 1838) (Tomlin, 1923: 311; Huber, 2010: 382). NHMUK 1907.10.28.124, one syntype (26.4 mm x 24.2 mm).

****quoyi, Cytherea*** – Hanley, 1844g: pl. 15, fig. 25 (without caption); 1846c: 11 (pl. expl.). Australia. This species has to date from 1846, i.e., the publication of the explanation to pl. 15. The Australian *Circe* (*Circe*) *quoyi* (Hanley, 1846) (Huber, 2010: 385, as "1844"). NHMUK 1907.10.28.207, syntype (48.4 mm x 47.3 mm).

roborata, Venus – Hanley, 1844g: pl. 16, fig. 25 (without caption); 1845b: 161; 1846c: 13 (pl. expl.); 1856a: 361. Van Diemen's Land [Tasmania, Australia]; Hanley & Metcalfe colls. Synonym of the Australian *Placamen placidum* (Philippi, 1844) (Lamprell & Whitehead, 1992: pl. 62, fig. 469). Philippi's species is the type species by original designation of *Placamen* Iredale, 1925. NHMUK 1907.10.28.115, one syntype (20.6 mm x 21.2 mm).

****scabra, Venus*** – Hanley, 1844g: pl. 16, fig. 24 (without caption); 1845b: 161; 1846c: 13 (pl. expl.); 1856a: 361. Catbalonga, Philippine Islands; Cuming & Hanley colls.; probably collected by H. Cuming. The Indo-Pacific *Timo-clea scabra* (Hanley, 1845) (Lynge, 1909: 244; Hylleberg & Kilburn, 2003: 220–221; Qi, 2004: 309–310, pl. 168, fig. H, the first and last as "1844"). NHMUK 1907.10.28.134–135, two syntypes (Higo et al., 2001: 176, fig. B1140). Lamprell (2004: 4) stated that this lot was the "holotype," but as it has two

specimens, this was not a valid lectotype designation. (Higo et al., 2001, mistakenly used the number of a different type, that of *Venus subnodosa* Hanley.)

***sculpta, Artemis** – Hanley, 1844g: pl. 15, fig. 42 (without caption); 1845c: 12; 1846c: 11 (pl. expl.); 1856a: 357. “Australia?”; Hanley coll. The Australian *Dosinia sculpta* (Hanley, 1845) (Lamprell & Whitehead, 1992: pl. 70, fig. 555). Type material not found in NHMUK or Leeds Museum in 2012.

simplex, Artemis – Hanley, 1845c: 11; 1844g: pl. 15, fig. 41; 1846c: 11 (pl. expl.); 1856a: 357. Panama & Santa Elena [Guayas, Ecuador]; Cuming & Hanley colls.; probably collected by H. Cuming. Synonym of the tropical eastern Pacific *Dosinia dunkeri* (Philippi, 1844) (Coan & Valentich-Scott, 2012: 825–826). Type material not found in NHMUK or Leeds Museum in 2012.

***subnodulosa, Venus** – Hanley, 1844g: pl. 16, fig. 19 (without caption); 1845b: 160; 1846c: 12 (pl. expl.); 1856a: 360. San Nicolas, Philippine Islands; Cuming & Hanley colls.; probably collected by H. Cuming. The western Pacific *Timoclea subnodosa* (Hanley, 1845) (Lynge, 1909: 243, pl. 5, fig. 5, as “1844”; Hylleberg & Kilburn, 2003: 221, as “1843”; Qi, 2004: 309, pl. 168, fig. D, as “1844”). NHMUK 1907.10.28.17–19, three possible syntypes (Higo et al., 2001: 176, fig. B1144).

***subquadrata, Artemis** – Hanley, 1844g: pl. 15, fig. 39 (without caption); 1845c: 11–12; 1846c: 11 (pl. expl.); 1856a: 357. Santa Elena, West Colombia [Ecuador]; Cuming & Hanley colls.; probably collected by H. Cuming. The tropical eastern Pacific *Cyclinella subquadrata* (Hanley, 1845) (Coan & Valentich-Scott, 2012: 823–824). NHMUK 1998196/1, lectotype (selected by Coan, 2001: 352, fig. 6); NHMUK 1998196/2, paralectotype.

varians, Cytherea – Hanley, 1844e: 109; 1844g: pl. 15, fig. 33; 1846c: 11 (pl. expl.); 1856a: 356. Brazil. Synonym of the western Atlantic *Pitar fulminatus* (Menke, 1828) (Rios, 1994: 286, pl. 98, fig. 1403). NHMUK 1912.6.4.15, figured syntype. The NHMUK collection has an additional three specimens from the Cuming collection; type status uncertain.

lamarckii, Venus – “Hanley”, 1843b: 113, ex Gray ms. Red Sea. This was treated as a separate, non-Gray species by Petit (2012: 72). However, it appears to be the same taxon as *Dosina lamarckii* Gray, 1838 (p. 308). Syn-

onym of the widely distributed Indo-Pacific *Antigona lamellaris* Schumacher, 1817 (Fischer-Piette, 1975: 37–38, as *Venus*; Oliver, 1992: 180, pl. 38, fig. 2; Huber, 2012: 363).

Two taxa in G. B. Sowerby II’s *Thesaurus Conchyliorum* monograph of the genus *Tapes* (1852) were indicated as being by Hanley, one marked “ms”, the other not. Both should be considered as being by G. B. Sowerby II, ex Hanley ms, not as Hanley in Sowerby. These are: *Tapes indica* – p. 694, pl. 151, figs. 146, 147 – and *T. variegata* – p. 696, pl. 151, figs. 133–138. Petit (2009) attributed these to G. B. Sowerby II.

Solenidae

acinaces, Solen – Hanley, 1843e: 101. Loc. unknown. The species was published without a figure, so it remains a *nomen dubium* (M. Huber, 20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

brevis, Solen – Hanley, 1842b: 12–13, pl. 2, fig. 2, ex Gray ms; 1843f: 2 (Syst. List), [2] (expl. to pl. 2), 8 (expl. to pl. 13); 1843g: pl. 13, fig. 42; 1856a: 336. “American Seas”. Synonym of the Indo-Pacific *Solen vagina* Linnaeus, 1758 (Cosel, 1990: 299). *Solen brevis* Hanley, 1842, is the type species of *Fistula* Mörch, 1853, by the subsequent designation of Keen (1969), making *Fistula* a synonym of *Solen*, because *S. vagina* is its type species. Type material not found in NHMUK or Leeds Museum in 2012.

***cylindraceus, Solen** – Hanley, 1843e: 101; 1843g: pl. 12, fig. 41; 1844g: 7 (pl. expl.); 1856a: 337. Loc. unknown. The western Indian Ocean *Solen cylindraceus* Hanley, 1843 (Cosel, 1990: 297). Type material not found in NHMUK or Leeds Museum in 2012.

***guinensis, Solen** – Hanley, 1842b: 12, pl. 2, fig. 1, ex Gray ms; 1843f: 2 (Syst. List), [2] (expl. to pl. 2), 8 (expl. to pl. 13); 1843g: pl. 13, fig. 28; 1856a: 336. Loc. not stated. The west African *Solen guinensis* Hanley, 1842. Unnecessarily renamed as *S. guinaicus* by Cosel (1993: 217–220) on the grounds that it was preoccupied by *Solen guineensis* W. Wood, 1815, but the one-letter difference in the species names prevents homonymy – ICZN Code Arts. 57.6). A neotype for Hanley’s species was designated by Cosel: NHMUK 1994154 (73.8 mm x 14.7 mm, from Dixinn Port, Conakry Peninsula, Guinea).

niveus, Solen – Hanley, 1843g: pl. 12, fig. 40 (without caption); 1846c: 7 (pl. expl.); 1856a: 336. Loc. not stated. This species remains a *nomen dubium* (M. Huber, pers. comm.,

20 Dec. 2011). Type material not found in NHMUK or Leeds Museum in 2012.

***philippinarum, Solen** – Hanley, 1843e: 101; 1843f: 7 (pl. expl.); 1843g: pl. 12, fig. 42; 1856a: 337. Philippine Islands. Although listed by Hidalgo (2003: 18–19) from the Philippine Islands, this seems to have been based on a mislocalized specimen and is a senior synonym of the tropical eastern Pacific *Solena rudis* (C. B. Adams, 1852); Huber (2010: 665) determined that the specimen subsequently illustrated by G. B. Sowerby II (1874: fig. 21b–21c) was the same specimen described and illustrated by Hanley, so Huber selected that specimen as the lectotype, with the remaining specimens in that lot becoming paralectotypes. All are from “Tacloban” (Philippines), but Huber corrected the type locality to western Panama. NHMUK 20120175/1, lectotype (126.2 mm x 25.2 mm) (selected by Huber, 2010: 665); NHMUK 20120175/2–3, two paralectotypes.

***sloanii, Solen** – Hanley, 1842b: 12, ex Gray ms; 1843f: 2 (Syst. List), 5 (expl. to pl. 11); 1843g: pl. 11, fig. 18; 1856a: 336. Loc. not stated. The Southeast Asian and Philippine Islands *Solen sloani* Hanley, 1842 (Cosel, 1990: 298; 2011: pl. 1180, figs. 1, 2, who attributed this species to Gray; Li, 2004: 275, pl. 151, fig. G). NHMUK 1952.5.13.1, two syntypes (Higo et al., 2001: 169, fig. B902, as “Gray, 1842” and erroneously identified as the “lectotype”). Wilkins (1953b: 43), in his catalogue of the Hans Sloane collection, stated: “Holotype BM 1952.5.13.1,” but this was not a valid lectotype selection as there were two syntypes in that lot, from the Sloane collection and labeled as from “Pegu, Lower Burma.”

***thuelcha, Solen** – Hanley, 1842b: 13, pl. 2, fig. 3, ex d’Orbigny ms; 1843f: 2 (Syst. List), [2] (expl. to pl. 2), 3 (expl. to pl. 10); 1843g: pl. 10, fig. 30; 1856a: 336. South America. Overlooked by Sherborn because of its attribution to d’Orbigny, who never published it. The southwestern Atlantic *Solen thuelchus* Hanley, 1842 (Cosel, 1990: 300). Misspelled as *S. tehuelchus* by Rios (1994: 267), who also attributed the species to d’Orbigny. Type material not found in NHMUK or Leeds Museum in 2012.

Pharidae

***acuminatus, Solen** – Hanley, 1842b: 17, pl. 2, fig. 29; 1843f: 2 (Syst. List), [2] (expl. to pl. 2), 5 (expl. to pl. 11); 1843g: pl. 11, fig. 35; 1856a: 336. The Indian Hooghley River, western

India [West Bengal]. Attributed to Reeve in Sherborn (1922: 57). Interpreted as *Pharella acuminata* (Hanley, 1842) (Huber, 2010: 669). NHMUK 20120174, three possible syntypes (Mus. Cuming, from Hooghley River; however, the tablet also lists “Singapore” which may have been added later).

***theobaldi, Novaculina gangetica** var. – Hanley & Theobald, 1874: 48, pl. 116, fig. 10, ex Benson ms. Tenasserim River, Pegu [Bago], Burma. This subspecies was never described by Benson, and it was not mentioned by Preston (1915). It might either be a synonym of *N. gangetica*, or it might well be a valid species, because species of *Novaculina* may have narrowly restricted distributions. Type material not found in NHMUK or Leeds Museum in 2012.

Myidae

***semistriata, Mya** – Hanley, 1842b: 20, pl. 2, fig. 42; 1843d: 6–7; 1843f: 3 (Syst. List), [2] (expl. to pl. 2), 3 (expl. to pl. 10); 1843g: pl. 10, fig. 16. Loc. unknown. The southeast Asian *Cryptomya* (*Venatomya*) *semistriata* (Hanley, 1842) (Zhang et al., 2012: 51). NHMUK 20120167, syntype (one broken valve).

Corbulidae

pygmaea, Corbula – Hanley, 1843f: 7 (pl. expl.); 1843g: pl. 12, fig. 34; 1856a: 344–345. Loc. not stated. The species dates from 1843g when the earlier plate explanation came to correspond with a published figure. Huber (2010: 771, as “1856”) treated this as a *nomen dubium*. *Corbula pygmaea* H. Adams, 1873, is a junior homonym. NHMUK 20120173, figured syntype (Cuming coll.).

quadrata, Corbula – “Hanley”, 1843f: 6 (Syst. List), 7 (expl. to pl. 12); Hinds, 1843: 57; Hanley, 1843g: pl. 12, fig. 36. The eastern Pacific and western Atlantic *Basterotia quadrata* (Hinds, 1843) (Coan & Valentich-Scott, 2012: 452–454, as “Hanley”). This name was credited to Hanley in the mistaken belief that it was made available by Hanley in early 1843. However, it was a *nomen nudum* in Hanley’s publications until the corresponding figure was published in “late” 1843; without an exact date, this has to be considered to have been at the end of the year, whereas the Hinds description can be dated as November 1843, and thus takes precedence. *Corbula*

quadrata “Hanley” Hinds, 1843, is type species of *Harlea* Gray, 1842; declared a *nomen oblitum* by Petit (2012: 100). Type material not found in NHMUK or Leeds Museum in 2012 for specimens of either Hinds or Hanley.

Pholadidae

clausus, Pholas – Hanley, 1842b: 6, pl. 1, fig. 34, ex Gray ms; 1843f: [1] (Syst. List), [1] (expl. to pl. 1), 4 (expl. to pl. 11); 1843g: pl. 11, fig. 8; 1856a: 336. Africa. Originally as “Gray in Bowdich” and repeated as such, but not a Gray species (Petit, 2012: 63). Synonym of the west African *Talona explanata* (Spengler, 1792) (Tryon, 1862: 207) (figured in Huber, 2010: 475). *Pholas clausa* Hanley, 1842, is the type species of *Talona* Gray, 1842, by the subsequent designation of Gray (1847). NHMUK 1952.10.30.168, syntype.

Teredinidae

****megotara, Teredo*** – Hanley, in Forbes & Hanley, 1848: 77–80, pl. 1, fig. 6, pl. 18, figs. 1, 2. Herne Bay, Kent; Torbay; Exmouth; Swansea Bay; Broadstairs [all United Kingdom]. This was presented as a new, more appropriate name for *Teredo nana* Turton, 1822, which Hanley felt was based on juvenile material. The type material for Turton’s species is in the USNM, and Turner (1966: 112) considered it too fragmentary to identify and Turton’s name thus a *nomen dubium*. Turner (1966: 110) misdated the text of Forbes & Hanley’s account as 1853. This is now regarded as the North Atlantic *Psiloteredo megotara* (Hanley, in Forbes & Hanley, 1848) (Turner, 1966: 110, pl. 25A, B). *Teredo megotara* Hanley, in Forbes & Hanley, 1848, is the type species of *Dactyloterredo* Moll, 1952, by original designation. NHMUK 1907.10.28.34, lectotype (selected by Turner, 1966: pl. 25A); NHMUK 1907.10.28.35, paralectotype.

utriculus, Teredo – “Hanley”, 1882a: 541, pl. 12, figs. 9–12; 1885a: 25–26. Cannes, France; from a shipwreck. “Non *T. utriculus* Gmelin, 1791”. Turner (1966: 128) interpreted Hanley’s taxon as a new species, with the earlier one by Gmelin being a *nomen dubium*, but because Hanley specifically referred to Gmelin, it is more appropriate to consider Hanley’s citation as a misidentification of

the earlier taxon, not a new homonymous species. Hanley’s own material was *Nototeredo norvagica* (Spengler, 1792). NHMUK 1907.12.30.589–591, Hanley’s specimens (four valves, two pallets, and two long tubes, all from Cannes, France) (figured by Turner, 1966: pl. 24, fig. E, but erroneously labeled as “holotype”).

Thraciidae

elongata, “Osteodoma” – Hanley, 1842b: 25, ex Gray ms; 1843b: pl. 3, fig. 16; 1843f: [3] (expl. to pl. 3), 8 (expl. to pl. 13); 1843g: pl. 13, fig. 27. Mediterranean. Synonym of *Lyonsia norvegica* (Gmelin, 1791) (Sabelli et al., 1990: 340). Type material not found in NHMUK or Leeds Museum in 2012.

Osteodoma – “Hanley”, 1842b: 24. Error pro *Osteodesma* Deshayes, 1825.

Periplomatidae

obtusa, Periploma – Hanley, 1842b: pl. 2, fig. 50 (without caption); 1843f: 3 (Syst. List), [2] (expl. to pl. 2, with descriptive footnote for this species), 8 (expl. to pl. 13); 1843g: pl. 13, fig. 50. America: “California?”. This species, which dates from the appearance of the explanation to pl. 3 in 1843f, is a synonym of the eastern Pacific *Periploma* (*Periploma*) *planusculum* G. B. Sowerby I, 1834 (Coan & Valentich-Scott, 2012: 994–995). NHMUK 1968360, holotype (46.2 mm x 32.5 mm). Hanley (1843f: [2]) stated “The shell from which this figure was copied,” indicating that the species was based on a unique specimen.

Pandoridae

****cumingii, Pandora*** – Hanley, 1861d: 272. Samar & Negros, Philippine Islands; in soft sand; H. Cuming. NHMUK 1964466, three syntypes.

GASTROPODA Haliotidae

sanguinea, Haliotis – Hanley, 1840 & 1842a: viii (fig. caption), 60 (description), frontispiece, fig. 5. This species was noted by Tomlin (1945:

91). Synonym of the South African *Haliotis spadicea* Donovan, 1808 (Geiger, 2000: 84–85, pl. 14, text fig. 70; Geiger & Owen, 2012: 126–127). NHMUK 1912.6.18.31, syntype (48.1 mm x 30.7 mm).

Fissurellidae

rosea, *Emarginula* – “Hanley”, 1844a: xxxii. Listed by Sherborn (1930: 5549) as also having been proposed by Hanley, but this is the species first made available by Bell (1824) (Forbes & Hanley, 1849: 479). This eastern Atlantic species was discussed and figured in Fretter & Graham (1976: 9–10, fig. 6).

Lepetidae

Pilidium – Forbes & Hanley, 1849: 440–442. *Non Pilidium* J. Müller, 1846 (Nemertea). Type species by monotypy: *Patella fulva* O. F. Müller, 1776. Synonym of *Iothia* Forbes, 1849, which has the same type species (Warén et al., 2011).

****Propilidium*** Forbes & Hanley, 1849: 443–445. Type species by monotypy *Patella ancyloides* Forbes, 1840. A valid genus (Keen, 1960: 236; Fretter & Graham, 1976: 34–36).

Neritidae

coluber*, *Nerita – Hanley & Theobald, 1875: 64, pl. 157, fig. 10, ex Thorp ms. Ceylon. Status uncertain. Tryon (1888: 53, pl. 17, fig. 78) transferred this to *Neritina*, without explanation. Species not included in Eldredge (1987). Probably a junior synonym of *Clithon oualaniensis* (Lesson, 1831) (C. Krijnen, pers. comm., 12 Aug. 2012). That polymorphic species is widely distributed across South Asia, and the color pattern of Hanley & Theobald's figured specimen falls within the range of color variation of *C. oualaniensis*, as documented by Grüneberg (1976, 1982). Type material not found in NHMUK or Leeds Museum in 2012.

Ampullariidae

****cerasum*, *Ampullaria*** – Hanley, 1854: *Ampullaria* pl. 2, fig. 7 (expl. on unnumbered p.). No loc. stated. *Pomacea cerasum* (Hanley, 1854), from Tabasco, Mexico (Cowie & Thiengo, 2003: 59; Thompson, 2011: 22). NHMUK 1907.10.28.225, syntype (24.3 mm x 22.4 mm).

****dysoni*, *Ampullaria*** – Hanley, 1854: *Ampullaria* pl. 2, fig. 5 (expl. on unnumbered p.). Honduras. *Pomacea flagellata dysoni* (Hanley, 1854) (Cowie & Thiengo, 2003: 62; Thompson, 2011: 23). NHMUK 1907.11.21.65, syntype (57.7 mm x 51.9 mm).

fasciata*, *Ampullaria globosa var. – Hanley & Theobald, 1874: 46, pl. 113, fig. 5. Moradabad [India], *non Ampullaria fasciata* Reeve, 1856. Synonym of *Pila globosa* (Swainson, 1822) (Prashad, 1923: 587, as *Pachylabra*). See also Subba Rao (1989: 58); Ramakrishna & Dey (2007) made no mention of Hanley & Theobald's name. Type material not found in NHMUK or Leeds Museum in 2012.

sphaerica*, *Ampullaria globosa var. – Hanley & Theobald, 1874: 46, pl. 113, fig. 4. Moradabad [India]. Synonym of *Pila globosa* (Swainson, 1822) (Prashad, 1923: 587, as *Pachylabra*). See also Subba Rao (1989: 58); Ramakrishna & Dey (2007) made no mention of Hanley & Theobald's name. Type material not found in NHMUK or Leeds Museum in 2012.

****theobaldi*, *Ampullaria*** – Hanley, in Hanley & Theobald, 1874: 47, pl. 115, fig. 2; Hanley, 1876: 605–606; “Birmah? or Pegu? the precise locality was mislaid” (Hanley & Theobald, 1874); Bhamao, Birmah; Theobald; Hanley coll. (Hanley, 1876). The Indian *Pila theobaldi* (Hanley, in Hanley & Theobald, 1874) (Prashad, 1923: 589, as *Pachylabra*). *Pila theobaldi* (Hanley, 1874) (Subba Rao, 1989: 60; Ramakrishna & Dey, 2007: 104). Mentioned from “Martaban” and “Moulmain” by Theobald (1876: 13, 52a). NHMUK 1907.10.28.1, possible syntype (69.2 mm x 73.7 mm) (The description makes clear that this was described from a single specimen, but there is a note with this lot, “specimen smaller than fig. in Conch. Ind.”, and the original size was given as 3.5 inches, corresponding to 90 mm).

swainsoni*, *Ampullaria – “Hanley,” 1854: *Ampullaria* pl. 1, figs. 1, 4. Alderson (1926) renamed this species *Ampullaria hanleyana* on the grounds that it was a junior homonym of *Ampullaria swainsoni* Philippi, 1852. However, because Hanley captioned his figure as “*Ampullaria swainsoni* of Philippi”, Alderson's taxon cannot be taken as the renaming of a homonym, but rather has to be interpreted as a new species based on Hanley's figure (Cowie & Thiengo, 2003: 66).

Cyclophoridae

anguis, *Cyclophorus stenostoma* var. – Hanley & Theobald, 1875: 43, pl. 105, fig. 9. Top of the Nilgherries. Synonym of *Theobaldius stenostoma* (G. B. Sowerby I, 1843) (Kobelt, 1902: 96; Gude, 1921: 43, as a variety; Ramakrishna et al., 2010: 46 (entry for *stenostoma* mentions that “Hanley & Theobald named a variety *anguis* also from Nilgris,” indicating that they regarded it a synonym). Type material not found in NHMUK or Leeds Museum in 2012; NHMUK has two uncatalogued specimens from the Nilgherries, ex H. Cuming, but not indicated as types or as from the Hanley collection.

assamensis, *Pterocyclos parvus* var. – Hanley & Theobald, 1875: 56, pl. 5, fig. 3. Khasia Hills: Assam. The illustrated specimen was originally identified as *P. parvus* by Hanley & Theobald (1870: 3, pl. 5, fig. 3), then later described as a new variety. Synonym of *Pterocyclos parvus* (Pearson, 1833) (Kobelt, 1902: 167; Gude, 1921: 109; Ramakrishna et al., 2010: 78, as a variety). Type material not found in NHMUK or Leeds Museum in 2012.

conica, *Cyclophorus tryblum* var. – Hanley & Theobald, 1872: 22, pl. 47, fig. 10. No locality; nominal species is from “Darjiling; Sikkim Himalayah.” Synonym of *Cyclophorus* (*Glossostylus*) *tryblum* Benson, 1854 (Kobelt, 1902: 120, as a synonym; Gude, 1921: 68–69, as a variety). Ramakrishna et al. (2010: 24–25) only mentioned *tryblum*, but not *conica*. Type material not found in NHMUK or Leeds Museum in 2012.

fulgurans, *Cyclophorus arthric[us]* var. – Hanley & Theobald, 1870: 2, pl. 3, fig. 2. [No locality; nominotypical name corrected to *arthriticus* by Hanley & Theobald, 1876: xv]. Synonym of *Cyclophorus* (*Salpingophorus*) *arthriticus* Theobald, 1864 (Kobelt, 1902: 124–125, as a synonym; Gude, 1921: 70, as a variety). Type material not found in NHMUK or Leeds Museum in 2012.

margarita, *Alycaeus – Hanley & Theobald, 1874: 39, pl. 95, fig. 10 [not pl. 97, fig. 7]; ex Theobald ms. Shan Provinces. *Alycaeus* (*Cycloryx*) *margarita* Hanley & Theobald, 1874 (Kobelt, 1902: 347; Gude, 1921: 211–212). The specimen figured on pl. 97, fig. 7 is *Alycaeus sculptilis* Benson, 1856 (*fide* Theobald, 1876: 52a; Godwin-Austen, 1914: 416). Type material not found in NHMUK or Leeds Museum in 2012.

mastersi, *Pterocyclos (Spiraculum) – Hanley & Theobald, 1870: 3, pl. 5, fig. 1, ex Blanford ms. Assam. “Golahgat, Assam” (*fide* Theobald, 1876: 38). *Pearsonia* (*Pearsonia*) *mastersi* (Hanley & Theobald, 1870) (Kobelt, 1902: 174; Gude, 1921: 120–121; Mitra et al., 2005: 85–86). Type material not found in NHMUK or Leeds Museum in 2012; NHMUK has two lots from the Blanford collection (one specimen from Assam, and three from the Naga Hills), but these specimens do not match the illustration and are probably not types.

ophis, *Cyclophorus – Hanley, 1876: 605; Hanley & Theobald, 1876: 57, pl. 144, fig. 6. Tenasserim; Theobald coll. *Cyclophorus* (*Salpingophorus*) *ophis* Hanley, 1876 (Kobelt, 1902: 130; Gude, 1921: 81). Type material not found in NHMUK or Leeds Museum in 2012.

picta, *Cyclophorus affinis* var. – Hanley & Theobald, 1874: 42, pl. 104, fig. 1. No locality. *Cyclophorus* (*Cyclophorus*) *affinis* Theobald, 1857 (Kobelt, 1902: 135; Gude, 1921: 89, both as a variety); Ramakrishna et al. (2010: 19) only mentioned *affinis* but not *picta*). Type material not found in NHMUK or Leeds Museum in 2012.

serratizona, *Cyclophorus – Hanley & Theobald, 1876: 57, pl. 144, fig. 7, ex Thorp ms. “Upper” Salwen (collected by Theobald). *Cyclophorus* (*Salpingophorus*) *serratizona* Hanley & Theobald, 1876 (Gude, 1921: 77). Theobald (1876: 53a) stated that the word “Upper” should be deleted from the type locality. Type material not found in NHMUK or Leeds Museum in 2012.

Viviparidae

ecarinata, *Paludina ceylanica* var. – Hanley & Theobald, 1874: 47, pl. 115, fig. 9. “Common in Ceylon.” A synonym of *Bellamya ceylonica* (Dohrn, 1857) (Starmühlner, 1974: 114). Preston (1915: 90) noted the type specimen was lost; type material not found in NHMUK or Leeds Museum in 2012.

listeri, *Paludina* – Forbes & Hanley, 1850: 8–11, pl. 71, fig. 16; 1852: 261. “Chiefly confined to the southern half of England” [many localities mentioned]. Synonym of *Viviparus contectus* (Millet, 1813) (Fretter & Graham, 1978a: 109–111, fig. 105). Type material not found in NHMUK or Leeds Museum in 2012.

Seven species in Reeve's *Conchologia Iconica* monograph of the genus *Paludina* (1862–1863) were based on Hanley manuscript names in the Cuming collection and should be treated as “Reeve, ex Hanley ms”, not as “Hanley in Reeve”. These include: *Paludina ampullarioides* – pl. 6, fig. 30; *P. cumingii* – pl. 3, fig. 11; *P. filosa* – pl. 6, fig. 31; *P. intermedia* – pl. 9, fig. 57; *P. thersites* – pl. 9, fig. 49; *P. viridis* – pl. 4, fig. 20; and *P. zonata* – pl. 6, fig. 34.

Pachychilidae

aspera, *Melania variabilis* var. – Hanley & Theobald, 1874: 44, pl. 109, fig. 6, ex Benson ms. Hindostan. Synonym of the southeast Asian *Brotia costula* (Rafinesque, 1833) (Köhler & Glaubrecht, 2006: 178). Hanley & Theobald (1874: 44) incorrectly cited “Benson 543, pl. 31, f. 12, 15.” This was a garbled citation of the treatment of *Melania variabilis* Benson, 1836 [*non* Defrance, 1823], in Souleyet (1852: 545, pl. 31, figs. 12–15). Leeds Museum 1957.173.1863, figured syntype (46.8 mm x 17.2 mm).

cincta, *Melania variabilis* var. – Hanley & Theobald, 1874: 44, pl. 109, fig. 5, ex Benson ms. Assam “(fide Bacon)”, *non* *Melania cincta* I. Lea & H. C. Lea, 1850 (India). Synonym of the Southeast Asian *Brotia costula* (Rafinesque, 1833) (Köhler & Glaubrecht, 2006: 178). NHMUK 1907.12.30.177, figured syntype (57.1 mm x 16.5 mm).

fimbriata, *Melania* – Hanley, 1854: Melaniadae pl. 4, fig. 32 (with expl. on unnumbered p.), ex Thorp ms. No loc. provided. Brot (1860: 110) treated this as a junior synonym of *Melania aspirans* Hinds, 1844; however, *M. aspirans* is now considered to belong to the Thiariidae, not the Pachychilidae, so the status of Hanley's species remains unresolved (M. Glaubrecht, pers. comm. 1 Aug. 2012). Type material not found in NHMUK or Leeds Museum in 2012.

fusiformis, *Melania baccata var. – Hanley & Theobald, 1873: 32, pl. 75, fig. 2. Shan States. Listed as valid in Preston (1915: 26). NHMUK 1907.12.30.210, figured syntype (63.0 mm x 25.1 mm).

“*Goliah*”, *Melania* – In 1873, Hanley & Theobald (1873: 31, pl. 72, fig. 3) wrote: “We had proposed the name of *Goliah* for this magnificent species, but . . . we defer the naming of it until the next part of our publication.” Three years

later, Hanley & Theobald (1876: 61, pl. 153, fig. 1), instead treated this as a synonym of *Melania reevei* Brot, 1862; and stated that: “the type in the British Museum is an immature form of the shell we had intended to call *Goliah* (pl. 72, f. 3) . . .” This name was thus not ever formally proposed, and was printed with a capital letter, in plain text, and not Latinized (in this work the Latin taxa were printed in boldface). As such, the specimen in NHMUK 1907.12.30.23, labeled as “holotype” (71.8 mm x 32.7 mm) (specimen figured on pl. 72, fig. 3), has no type status.

imbricata, *Melania reevei* var. – Hanley & Theobald, 1876: 61, pl. 153, fig. 4. Locality not stated. Synonym of *Brotia herculea* (Gould, 1846) (Köhler & Glaubrecht, 2006: 192) from Thailand. Type material not found in NHMUK or Leeds Museum in 2012.

limborgi, *Melania – Hanley, 1879: 580. Mulé-it Range, Tenasserim [Burma]; Limborg. Preston (1915: 29–30, fig. 2) listed this as *Tiara (Pachychilus) limborgi*, and illustrated the “type” specimen (his fig. 2), but this is not a valid lectotype designation as the original description referred to “specimens” and “all the examples” of this species. NHMUK 1907.10.28.228–229, syntypes (2 shells from Tenasserim, one illustrated by Preston); NHMUK 1905.03.7.1, syntypes (2 shells from Moulmein, Tenasserim, ex Godwin-Austen).

peguensis, *Melania reevei* var. – Hanley & Theobald, 1873: 31, pl. 72, fig. 6, ex Anthony ms. Pegu. Hanley & Theobald (1876: xvi, footnote 11) explained that they had initially believed that Anthony had published this species in the *American Journal of Conchology*, but they subsequently realized that it was merely “a manuscript species.” Preston (1915: 22), listed it as var. of *Tiara (Melanoides) gloriosa* (Anthony, 1865). Köhler & Glaubrecht (2006: 192) listed it as a *nomen nudum*, but they cited Hanley & Theobald's figure, showing that it was made available. It is presumably a synonym of *Brotia herculea* (Gould, 1846). NHMUK 1907.12.30.25, figured syntype (75.3 mm x 25.8 mm).

sowerbii, *Melania herculea var. – Hanley & Theobald, 1874: 44, pl. 109, fig. 7, ex Gould ms. Tenasserim [Burma]. NHMUK 1907.12.30.176, probable syntype (71.8 mm x 27.3 mm) (slightly smaller than illustrated specimen).

spinosa, *Melania* – Hanley, 1854: Melaniadae pl. 1, fig. 7, ex Benson ms; Hanley & Theobald, 1873: 32, pl. 75, fig. 6. *Non* *M.*

spinosa Gray, 1824. River Jumna, Sylhet, British India [Chittagong Province, Bangladesh]. Synonym of the southeast Asian *Brotia costula* (Rafinesque, 1833) (Köhler & Glaubrecht, 2006: 178). NHMUK 1907.10.28.79, lectotype (selected by Köhler & Glaubrecht, 2002: 148); NHMUK 1907.10.28.80, paralectotype.

lanceolata, *Melania reevei* var. – “Hanley & Theobald, 1876”. This subspecies was first described by Nevill (1885: 248–249), who cited Hanley & Theobald (1876: pl. 153, fig. 1) for the illustration. Preston (1915: 23) erroneously attributed this species name to Hanley & Theobald, not to Nevill.

schomburgki, *Melania* – “Hanley”. Reeve, 1859: pl. 14, fig. 93, ex Hanley ms. This species was indicated by Reeve as having been taken from a Hanley manuscript. Locality unknown; Cuming collection.

Paludomidae

melanostoma*, *Paludomus – Hanley & Theobald, 1875: 49, pl. 121, figs. 8–9, ex Thorpe ms. Ceylon [Sri Lanka]. Synonym of *Paludomus* (*Tanalia*) *neritoides* Reeve, 1847 (Starmühlner, 1974: 150; 1977: 263). NHMUK 1907.12.30.85, figured syntype (25.1 mm x 21.6 mm).

****monile*, *Paludomus*** – Hanley & Theobald, 1874: 44, pl. 108, fig. 10, ex Thorpe ms. Southern India. A *Paludomus* of unresolved status. Preston (1915: 48) and Satyamuri (1960: 48–49) considered it a valid species; Theobald (1876: 9) considered it a synonym of *P. chilinoides* Reeve, 1847; and Brot (1880: 43–44) considered it a synonym of *P. obesus* Philippi, 1847. NHMUK 1907.10.28.91, figured syntype (12.4 mm x 9.2 mm).

Thiaridae

****jugicostis*, *Melania*** – Hanley & Theobald, 1874: 45, pl. 110, figs. 8, 9, ex Benson ms. Tenasserim River. Listed as valid by Preston (1915: 28–29). *Melanoides jugicostis* (Hanley & Theobald, 1874) (Brandt, 1974: 166–167, 368, pl. 12, fig. 13) from the Tenasserim River, Burma. Now classified in the Thiaridae (M. Glaubrecht, pers. comm., 1 Aug. 2012). Type material not found in NHMUK or Leeds Museum in 2012.

Littorinidae

fairbanki*, *Cremnoconchus – Hanley & Theobald, 1876: 58, pl. 146, fig. 7, ex Blanford ms; Blanford, 1881: 221. Locality not stated. Junior synonym of *Cremnoconchus canaliculatus* Blanford, 1870 (D. Reid, pers. comm., 25 June 2012). Type material not found in NHMUK or Leeds Museum in 2012.

gracilior*, *Lacuna – Hanley, 1844a: viii, xxxviii, fig. 86, ex Metcalfe ms; Forbes & Hanley, 1850: 63–67, as a synonym of the eastern Atlantic *Lacuna vincta* (Montagu, 1803). Type material not found in NHMUK or Leeds Museum in 2012.

patula*, *Littorina – Thorpe, 1844: 259; Hanley, 1844a: [v], xxxix, fig. 7. Thorpe (1844: 259) had a description, and credited the name to Jeffreys, but it is not a known Jeffreys taxon (Warén, 1980: 21). In Hanley's Systematic Index, no description was present, and Thorpe's species was listed as a questionable variety of *Littorina rudis* (Maton, 1797) [now considered to be a synonym of *L. saxatilis* (Olivi, 1792)], suggesting that Hanley doubted its validity. Reid (1996: 264, 265) treated this taxon as a *nomen dubium* to ensure stability and to preserve the name *Littorina arcana* Hannaford Ellis, 1978. There is no known type material of Thorpe's species, and new material from the type locality was found to consist of a mixture of *Littorina saxatilis* and *L. arcana* (Hannaford Ellis, 1979: 52–53). The figure in Thorpe's book is insufficient to establish the identity of the species, because for these species of *Littorina*, information on reproductive mode is required for unequivocal identification (Hannaford Ellis, 1979; Reid, 1996). Thorpe's species is a senior homonym of *L. patula* Gould, 1849. The name *Littorina keenae* Rosewater, 1978, is the replacement name now in use for Gould's name.

Pomatiidae

partioti*, *Cyclostoma – Hanley, 1858f: *Cyclostoma* pl. 5, fig. 110 (expl. on unnumbered p.), ex Thorpe ms. No locality stated. *Non Cyclostoma* (*Pomatias*) *partioti* Saint-Simon, 1848, the latter now *Cochlostoma partioti* (Saint-Simon, 1848), which occurs in the Pyrenees. Type material not found in NHMUK or Leeds Museum in 2012.

****sowerbyi*, *Cyclostoma*** – Hanley, 1858f: *Cyclostoma* pl. 5, fig. 141 (expl. on unnumbered

p.). No loc. stated. Hanley speculated that the specimen he figured might be *C. bourcier* Pfeiffer, 1854 (p. 151), from Ecuador (which he misspelled as “*bouciera*”), but in case it was not that, the name *sowerbyi* was offered. Type material not found in NHMUK or Leeds Museum in 2012.

valvatoides, *Cyclostoma – Hanley, 1858f: *Cyclostoma* pl. 1, figs. 21, 22 (expl. on un-numbered p.), ex Thorpe ms. No loc. stated. Type material not found in NHMUK or Leeds Museum in 2012.

Naticidae

australis, “*Sicaretus*” [sic; *Sigaretus*] – Hanley, 1840 & 1842: viii (figure caption), 57–58, frontispiece, fig. 3. South Seas. Hanley’s (1842a: 153) Index credited this species to “Gray”, but it is not a Gray species. This species was overlooked by both Tomlin (1945) and by Sherborn. Synonym of the Indo-Pacific *Sinum laevigatus* (Lamarck, 1822) according to Tryon (1886: 55, pl. 24, fig. 50). NHMUK 1907.10.28.125, figured syntype.

Rissoidae

beanii, *Cingula – Hanley, 1844a: vi, xli–xlii, fig. 43 – on p. vi as “*Rissoa (Cingula)*”; Forbes & Hanley, 1850: 84–86, pl. 79, figs. 5, 6; 1852: 263, both as *Rissoa beanii*. Scarborough, U.K. *Alvania beanii* (Hanley, 1844) (Fretter & Graham, 1978b: 173–175, fig. 148, as “*beani* Thorpe”; Sabelli et al., 1990: 149; S. M. Smith & Heppell, 1991: 18; Warén, 1996: 225, fig. 17D, H). Type material not found in NHMUK or Leeds Museum in 2012.

calathus, *Rissoa* – Forbes & Hanley, 1850: 82–84, pl. 78, fig. 3. Herm, Hanley; Shellness, Kent, & Whitesand Bay, Jeffreys; Penzance, 15–20 fms. [27–55 m], M’Andrew & Forbes; Mizen Head, 50 fms. [91 m], M’Andrew; Kilkee, Clare, Ireland, W. Thompson, collected by Mr. Warren. Synonym of *Alvania beanii* (Hanley, 1844) (Fretter & Graham, 1978: 173; Sabelli et al., 1990: 149), or as a subspecies thereof (S. M. Smith & Heppell, 1991: 18, attributed to Hanley alone). SMNH 3868, syntypes. Additional type material not found in NHMUK or Leeds Museum in 2012. USNM 183454 (one specimen) is from Hanley, but without locality or indication that it might be a type specimen. USNM 183434, from the Jeffreys collection, is labeled as the “figured type,” but this is merely the specimen figured

by Jeffreys in his *British conchology* (1869: pl. 66, fig. 4).

scalariformis, *Rissoa* – Hanley, 1844a: viii, xlii, fig. 89, ex Metcalfe ms (on p. viii as *Cingula*); Forbes & Hanley, 1850: 78–80, as a synonym of *Rissoa zetlandica* (Montagu, 1815). Herm, near Guernsey, U.K. Senior primary homonym of the tropical eastern Pacific *R. scalariformis* C. B. Adams, 1852, for which the synonymous *Rissoina firmata* (C. B. Adams, 1852) proved to be available. *Alvania zetlandica* (Montagu, 1815) was figured by Fretter & Graham (1978b: 180–182, figs. 154, 155). Type material not found in NHMUK or Leeds Museum in 2012.

abyssicola, *Rissoa* – “Forbes & Hanley”. This species was so listed by Norris & Dance (2002: 369), but this species was indicated as being by Forbes alone in Forbes & Hanley (1850: 856 – May; pl. 78, figs. 1, 2 – Feb.), as other authors have correctly used it in recent years (Fretter & Graham, 1978b: 188–190, figs. 162–164).

Assimineidae

***globularis, “*Cingula?*”** – Hanley, 1844a: viii, xlii, fig. 87, ex Metcalfe ms. Weymouth, U.K. This was listed as a synonym of *Boreocingula globulus* (Möller, 1842) (S. M. Smith & Heppell, 1991: 19), but this is unlikely because that species occurs only in southwestern Greenland (Warén, 1996: 214–215). Jeffreys (1869: 102) listed it as a synonym of *Paludinella littorina* (Della Chiaje, 1828), and indeed, *Cingula globularis* Hanley, 1844, is the oldest valid name for the type species of *Paludinella* L. Pfeiffer, 1841, which was established by the subsequent designation of Herrmannsen (1847) as “*Helix littorina* Delle Chiaie, 1828”. This species is now understood to have been intended by Pfeiffer to correspond to the concept of this species *sensu* Philippi (1841), that is, referable to the Assimineidae, and not the original concept of Delle Chiaie, which was referable to the Littorinidae (Kadolsky, 2012: 66–69; ICZN Code Art. 70.3). For a treatment of this assimineid species, but without mention of Hanley’s taxon, see Fretter & Graham (1978a: 148–149, figs. 129, 130). Type material not found in NHMUK or Leeds Museum in 2012.

littorea, *Rissoa* – Forbes & Hanley, 1850: 132–134. Unjustified emendation of *Helix littorina* Della Chiaie, 1828 (fide Kadolsky, 2012: 69).

Bithyniidae

modesta*, *Bythinia – Hanley, 1859c: 240, ex Dohrn ms, *nomen nudum*. Ceylon.

Hydrobiidae

pellucida*, *Cingula – Hanley, 1844a: xliii–xliv, ex Bean ms; Forbes & Hanley, 1850: 140–141, as a synonym of *Rissoa ventrosa* (Montagu, 1803) based on a colorless specimen. Locality within the United Kingdom not stated. Figured by Fretter & Graham (1978a: 125–127, fig. 114), as *Hydrobia ventrosa*, now classified as the eastern Atlantic brackish water *Ecrobia ventrosus* (Montagu, 1803). Type material not found in NHMUK or Leeds Museum in 2012.

Lithoglyphidae

kingii*, *Natica – Forbes & Hanley, 1851: 343–344, pl. 101, figs. 1, 2. From the bottom of a boat that had been fishing a few miles off Cullercoats, Northumberland [United Kingdom], in the coralline zone, King. According to Jeffreys (1862: 70): “*Natica kingii* of Forbes and Hanley ... belongs to ... [the Paludinidae], and not to the Naticidae. It is ... *Lithoglyphus naticoides* ... [(C. Pfeiffer, 1828)], and inhabits the Danube. Professor King is said to have found the specimen (which is now in my collection) in the bottom of a fishing-boat at Cullercoats. How this Austrian and freshwater species could have got to the Northumberland coast, is very difficult to say. Professor King informs me that he never received any shells from the Danube, and that his statement as to the Northumbrian locality is perfectly correct. The question of the indigenesness and unaccountable habitat of this specimen must therefore remain a mystery.” Currently regarded as a synonym of *Lithoglyphus naticoides* (C. Pfeiffer, 1828). USNM 188241, holotype (6.2 mm x 5.3 mm).

Iravadiidae

****proxima*, *Rissoa*** – Thompson, 1847: 174, ex Alder ms, *nomen nudum*; Forbes & Hanley, 1850: 127, pl. 75, figs. 7, 8, ex Alder ms; 1852:

264. Cork Harbour, Bantry Bay, Portmarnock and Dublin Bay, Ireland; Jeffreys. Type species by monotypy of *Ceratia* H. Adams & A. Adams, 1852. The western Atlantic *Ceratia proxima* (Forbes & Hanley, 1850) (Ponder, 1984: 52–54); figured as *Onoba proxima* by Fretter & Graham (1978b: 167–169, figs. 143, 144). NHMUK 1904.12.30.546–548, 2 syntypes, including figured specimen (a third specimen is missing as of June 2012).

Tonnidae

angusta*, *Dolium variegatum var. – Hanley, 1860b: 491. Locality not stated. Based on a figure in Reeve (1848: pl. x, fig. 7b). Junior synonym of *Tonna variegata* (Lamarck, 1802) (Vos, 2007: 103–104). NHMUK 1968305, possible holotype (86.9 mm x 65.4 mm).

****dunkeri*, *Dolium*** – Hanley, 1860a: 431; 1860b: 490. Natal; Cuming coll. *Tonna dunkeri* (Hanley, 1860) (Kilias, 1962: 35; Vos, 2007: 93–94, pl. 41). NHMUK 1967677/1, lectotype (36.8 mm x 27.4 mm) (selected by Vos, 2007: 93); NHMUK 1967677/2–3, 2 paralectotypes.

favannii*, *Dolium – Hanley, 1860a: 430; 1860b: 490, as “*favannei*”. Locality unknown; Cuming coll. Unknown *Tonna* (Kilias, 1962: 36); synonym of the western Pacific *Tonna luteostoma* (Küster, 1857) (Vos, 2007: 96–97, pls. 43, 44, & 63, fig. 4. NHMUK 1967678, two syntypes (70.6 mm x 61.5 mm; 72.2 mm x 62.5 mm).

latesulcatum*, *Dolium – Hanley, 1860b: 489, ex Chemnitz (1777: 396, figs. 1072, 1082). Junior synonym of *Tonna allium* (Dillwyn, 1817) (Vos, 2007: 42). Type material presumably the specimen figured by Chemnitz.

picta*, *Dolium latesulcatum var. – Hanley, 1860b: 489. Locality not stated; Cuming coll. Junior synonym of *Tonna allium* (Dillwyn, 1817) (Vos, 2007: 42). Type material not found in NHMUK or Leeds Museum in 2012.

reevii*, *Dolium – Hanley, 1860b: 493. Locality unknown; Cuming coll. Synonym of the Indo-Pacific *Tonna lischkeana* (Küster, 1857) (Kilias, 1962: 26; Vos, 2007: 45–47, pls. 9, 10, & 60, figs. 1, 2, text fig. 11). NHMUK 196804, two syntypes (122.6 mm x 102.2 mm; 115.9 mm x 89.9 mm). Winckworth & Tomlin (1933: 212) had noted the existence of the syntypes but did not mention a catalogue number.

****tankervillii*, *Dolium variegatum*** var. – Hanley, 1860b: 490–491. Locality not stated. *Tonna tankervillii* (Hanley, 1860), a valid

species from eastern Australia and the North Island of New Zealand (Vos, 2007: 102–103, pls. 50–52). Leeds Museum 1957.173.29077, holotype (108.1 mm x 84.6 mm). Vos (2007: 102–103) stated that the specimen in the Leeds Museum was the “lectotype.” However, the original description did not encompass multiple specimens, but instead referred to a specimen “now in my own possession.” As no other syntypes are known, this specimen is here treated as the holotype.

tenebrosa, *Dolium galea var. – Hanley, 1860b: 488. Red Sea; Cuming collection & “elsewhere”. As a synonym of *T. galea* (Kilias, 1962: 38); *Tonna tenebrosa* (Hanley, 1860) from the Red Sea (Vos, 2007: 65, pls. 28, 61). NHMUK 20060062, syntype. Vos (2007: 65) erroneously stated that this specimen was a “neotype,” but it is from the Cuming collection, and the original description refers to two specimens, including one from the Cuming collection (the other syntype was not found in NHMUK or Leeds Museum in 2012).

cumingii, *Dolium* – “Hanley”. Reeve, 1849: pl. 8, fig. 13, ex Hanley ms; Hanley, 1860b: 491, as “*Dolium cumingii* Hanley”. Reeve indicated that this was a Hanley manuscript name, and it is therefore not “Hanley in Reeve”, as some authors have had it. Philippine Islands; Cuming. A valid Indo-Pacific species of *Tonna* (Vos, 2007: 79–80, pl. 35 & pl. 62, figs. 2, 3, as “Hanley in Reeve”). Leeds Museum 1857.173.29073, lectotype (92.4 mm x 69.0 mm) (selected by Vos, 2007: 75); Leeds Museum 1857.173.29074, paralectotype (34.0 mm x 25.8 mm).

Epitoniidae

fragilis, *Scalaria* – Hanley, 1840 & 1842: viii (figure caption), 63 (text fig.). Type locality not stated. This species was listed by Tomlin (1945: 91) but not by Sherborn. Hanley's (1842a: 153) Index credited this species to “Gray”, but it is not a Gray species. Possible synonym of *Scalaria eburnea* Potiez & Michaud, 1838 (Tryon, 1887: 71, pl. 14, fig. 57), which may or may not be from the western Atlantic (Clench & Turner, 1951: 288). Not “*Scalaria fragilis* Hanley”, of G. B. Sowerby II, 1844, a synonym of a different western Atlantic species (Clench & Turner, 1951: 260–261). NHMUK 1907.10.28.81–84, four syntypes.

Janthinidae

britannica, *lanthina* – Forbes & Hanley, 1852: 260, ex Jeffreys ms, thence ex Leach ms. In synonymy with *I.* [= *Janthina*] *communis* Lamarck, 1822, now regarded as a synonym of *Janthina janthina* (Linnaeus, 1758) (Fretter & Graham, 1982: 392–393, fig. 279). It would not be an available name under ICZN Code Art. 11.6.

Eulimidae

gracilis, *Eulima distorta* var. – Forbes & Hanley, 1850: 233 (Aug.), pl. 92, fig. 6. Clyde. *Non Eulima gracilis* C. B. Adams, 1850 (April), nor *Eulimella acicula gracilis* Jeffreys, 1847. Synonym of the eastern Atlantic *Melania monterosatoi* (Monterosato, 1890, ex Boury ms), proposed as a new name for *Eulima distorta gracilis* “Jeffer.”, non *E. gracilis* C. B. Adams, 1850 (Warén, 1988: 19–20, figs. 11, 12). Discussed but not figured in Fretter & Graham (1982: 420–421, as “1853” and as *Polygireulima*). SMNH 3610, syntypes. Additional type material not found in NHMUK or Leeds Museum in 2012.

Cerithiopsidae

***Cerithiopsis** Forbes & Hanley, 1850: pls. OO, 91; 1851: 364. Type species by monotypy: *Murex tubercularis* Montagu, 1803. This genus was first made available in the plate captions (published in 1850), a year before the text was published.

clarkii, *Cerithiopsis – Forbes & Hanley, 1851: 368, as “pl. 105, fig. 1”, but actually pl. 103, fig. 6. Exmouth; William Clark. Described as a possible synonym of *C. tubercularis* Montagu. Treated by Jeffreys (1867: 266–268) as an aberrant form of *C. tubercularis* with two rather than three rows of nodes; not mentioned by Fretter & Graham (1982: 366–368, figs. 257, 258) in their treatment of Montagu's species. On the other hand, Sabelli et al. (1990: 179) listed it in another genus of the Cerithiopsidae as *Dizoniopsis clarkii* (Forbes & Hanley, 1851). Type material lost in 1941 bombing of the Liverpool Museum (McMillan, 1997).

Buccinidae

nassoides, *Pseudoliva – Hanley, 1860a: 430; Hanley & Theobald, 1876: xvi, footnote 9, in the Melanidae. Malabaricum [Malabar,

India]; Hanley coll. This is not a member of the Pseudolividae and thus was not listed in Vermeij (1998). Hanley & Theobald (1876) stated that this was a junior synonym of the brackish-water *Clea annesleyi* Benson, 1860 (pp. 258–259), then classified in the Melanoidae. However, Benson's description was published on 1 October 1860, while Hanley's description would have been published no later than February 1860, so Hanley's name has priority over Benson's. In 1876, Brot transferred *Clea* to the Buccinidae, as discussed by E. A. Smith (1895). NHMUK 1907.10.28.236–237, two syntypes (7.3 mm x 4.0 mm; 8.3 mm x 4.5 mm). Benson's type material is not present in the NHMUK. Hanley's syntypes are juveniles, and it remains to be determined whether this species belongs to *Clea* (Buccinidae) or *Nassodonta* (Nassariidae), both brackish-water taxa.

Harpidae

****ponderosa*, *Oniscia*** – Hanley, 1858e: 255–256, pl. 42, figs. 9, 10. Locality unknown; Cuming coll. The southwestern Pacific *Morum ponderosum* (Hanley, 1860) (Poppe et al., 1999: pl. 39, figs. 2–4). Type species by original designation of *Herculea* H. Adams & A. Adams, 1858, a synonym of *Morum*. NHMUK 1966724, lectotype (32.7 mm x 25.8 mm) (selected by Dance & Emerson, 1967: 94); NHMUK 1966725, paralectotype.

Herculea “Hanley” – Evidently based on a Hanley manuscript name, this generic name was made available by H. Adams & A. Adams (1858: 621).

Volutidae

Cochlycopa Hanley, 1841: 27 [not to be confused with *Cochlicopa* Férussac, 1821, a pulmonate, which has been misspelled by some authors as *Cochlycopa*]. Type species by monotypy: *Achatina maculata* Swainson, 1821. Described as a subgenus of *Achatina*, a terrestrial pulmonate from Africa. However, Hanley (1841: 27–28) also noted that the Danish malacologist H. H. Beck (1799–1863) concluded that this species “however, much in appearance it may resemble a land snail, is an operculated marine mollusc and found alive in the seas of Spain and Portugal,” and if

Beck was correct, “the *Cochlycopa maculata* must form a genus of which it is the sole species, akin on the one hand to *Struthiolaria*, on the other to certain *Buccinums*.” This genus is a junior synonym of *Ampulla* Röding, 1798, and *Halia* Risso, 1826, which have, through synonymy, the same type species (Sabelli et al., 1990: 210; ICZN Opinion 968, 1971).

Pseudolividae

****ancilla*, *Pseudoliva*** – Hanley, 1860a: 429–430. Caffrariam [Eastern Cape, South Africa]; Hanley coll. The South African *Fulmentum ancilla* (Hanley, 1859) (Vermeij, 1998: 60, 80). Type species of *Mariona* G. B. Sowerby III, 1890, by monotypy, and of *Sylvanocochlis* Melvill, 1903, by original designation, both synonyms of *Fulmentum* Fischer, 1884. NHMUK 1910.5.26.12, holotype (44.0 mm x 22.9 mm).

Mangeliidae

[formerly part of Turridae]

metcalfei*, *Pleurotoma – Hanley, 1844a: xlvii–xlviii, ex Reeve ms; Forbes & Hanley, 1851: 485–488, as a synonym of *Mangelia costata* (Pennant, 1777). Guernsey, U.K.; Metcalfe coll. Mistakenly listed by Sherborn (1928: 4027) and by Norris & Dance (2002: 372) in *Parthenia*, and credited to “Thorpe” by Tucker (2004: 615). Synonym of *Mangelia costata* (Pennant, 1777). This European species was figured, as *Cytharella coarctata* (Forbes, 1840), another synonym of *Mangelia costata*, by Fretter & Graham (1988: 529–530, fig. 365). Type material not found in NHMUK or Leeds Museum in 2012.

Cancellariidae

Cancellariidae – Forbes & Hanley, 1851: 360, as Cancellariadae.

Architectonicidae

In his 1862 paper, Hanley did not spell out the genus into which he placed his new species, the only clue being the paper's title, “... new Solaria”, which is the vernacular plural of *Solarium*. Norris & Dance (2002) placed the contained species into the Latinized “*Solaria*”, which has to be taken as an incorrect subsequent spelling of *Solarium* Lamarck, 1799, a junior synonym of *Architectonica* Röding, 1798. *Solaria* Champion, 1908, is a genus of beetles.

australis, *Solarium (Philippia) hybridum* var. – Hanley, 1863a: 236. Loc. not stated, but presumably Australia. *Non Solarium australe* Philippi, 1849, which is a junior synonym of *Architectonica perspectiva* (Linnaeus, 1758). A synonym of the Indo-Pacific *Psilaxis radiatus* (Röding, 1798) or *Psilaxis oxytropis* (A. Adams, 1855) (Bieler, 1993: 117–125). Type material not found in NHMUK or Leeds Museum in 2012.

bairdii, *Solarium* – Hanley, 1863a: 231, pl. 254, figs. 48, 49. Loc. unknown; “British Museum” coll. *Nomen dubium* (Bieler, 1993: 336). Type material not found in NHMUK or Leeds Museum in 2012.

canaliferum, *Solarium* – Hanley, 1863a: pl. 254, figs. 98–100 (caption), ex [A.] Adams ms. Philippine Islands; Cuming coll. Although published into the synonymy of *S. cylindraceum* (Dillwyn, 1817), of Hanley, because of its subsequent use by authors, it is an available name. Synonym of the Indo-Pacific *Heliacus (Teretropoma) infundibuliformis* (Gmelin, 1791) (Bieler, 1993: 260–264). Type material not found in NHMUK or Leeds Museum in 2012.

cumingii, *Solarium* – Hanley, 1862: 204; 1863a: 232, 247, pl. 253, figs. 44, 45. Loc. unknown; Cuming coll. Synonym of the Indo-Pacific *Architectonica perspectiva* (Linnaeus, 1758) (Bieler, 1993: 38–45). NHMUK 1981157, holotype (21.8 mm x 34.9 mm).

dunkeri, *Solarium* – Hanley, 1862: 204; 1863a: 233, 247, pl. 252, figs. 29, 30. East Indies; Cuming & Hanley colls. Synonym of the Indo-Pacific *Architectonica perdix* (Hinds, 1844) (Bieler, 1993: 48–52). NHMUK 1981158, lectotype (20.2 mm x 30.6 mm, Cuming collection) (selected by Bieler, 1993: 52). Additional syntypes (which would be paralectotypes) not located in NHMUK or Leeds Museum in 2012.

junior, *Solarium (Torinia) stramineum* var. – Hanley, 1863a: 242. Loc. not stated. Synonym of the Indo-Pacific *Heliacus (Graneliacus) stramineus* (Gmelin, 1791) (Bieler, 1993: 255–259). Type material not found in NHMUK or Leeds Museum in 2012.

latic, *Solarium (Torinia) cyclostomum* var. – Hanley, 1863a: 239. Loc. not stated. Synonym of the Western Atlantic *Heliacus cylindricus* (Gmelin, 1791) (R. Bieler, pers. comm., 18 Sept. 2012). Type material not found in NHMUK or Leeds Museum in 2012.

planulata, *Solarium (Torinia) perspectivunculus* “var.?” – Hanley, 1863a: 238, pl. 254, fig. 63. Loc. not stated. Synonym of the Indo-Pacific *Heliacus (Heliacus) variegatus*

(Gmelin, 1791) (Bieler, 1993: 185–191). NHMUK 1907.10.28.64, figured syntype (6.7 mm x 12.7 mm).

reevei, *Solarium – Hanley, 1862: 204–205; 1863a: 234, 247, pl. 250, figs. 9, 10. Loc. unknown (1862), Sydney, Australia (1863a); Reeve coll. *Adelphotectonica reevei* (Hanley, 1862) (Bieler, 1993: 99–103) from Australia and the western Pacific. *Solarium reevei* Hanley, 1862, is the type species of *Adelphotectonica* Bieler 1987, by original designation. NHMUK 198049, holotype (17.7 mm x 22.7 mm).

regium, *Solarium* – Hanley, 1862: 205. Loc. unknown; Cuming coll. *Incertae sedis* (Bieler, 1993: 97–98). NHMUK 1981159, holotype (14.5 mm x 29.9 mm).

soverbii, *Solarium* – Hanley, 1862: 206; 1863a: 243, 247, pl. 254, figs. 81, 82. Tunetana [Tunisia]; British Museum. Listed by Norris & Dance (2002: 374) as “sowerbii”. Synonym of the eastern Atlantic *Pseudotorinia architae* (O. G. Costa, 1841) (Bieler, 1993: 277–281). Rendered as “sowerbii” by Monterosato (1873:11) and Marshall (1887: 32). NHMUK 1847.9.10.24–25, two syntypes (larger 6.2 mm in diameter, as measured by Bieler).

strigata, *Solarium (Torinia) infundibuliforme* “var.?” – Hanley, 1863a: 243, pl. 254, fig. 94. Synonym of the Indo-Pacific *Heliacus (Teretropoma) infundibuliformis* (Gmelin, 1791) (Bieler, 1993: 260–264). NHMUK 1907.10.28.62, figured syntype (6.3 mm x 10.7 mm).

taylori, *Solarium – Hanley, 1862: 205–206; 1863a: 230, 248, pl. 252, figs. 31, 32. Loc. unknown (1862); Zanzibar (1863a); Hanley coll. The western Pacific *Architectonica taylori* (Hanley, 1862) (Bieler, 1993: 71–73). NHMUK 1907.10.28.98, holotype (21.3 mm x 35.7 mm).

undata, *Solarium (Philippia) hybridum* var. – Hanley, 1863a: 236, pl. 253, figs. 42, 43. Loc. not stated; Taylor coll. Synonym of the Indo-Pacific *Psilaxis radiatus* (Röding, 1798) (Bieler, 1993: 117–125). Type material not found in NHMUK or Leeds Museum in 2012.

vermetiformis, *Solarium (Torinia) cylindraceum* var. – Hanley, 1863a: 243, pl. 254, fig. 100. Philippine Islands; Cuming coll. Synonym of the Indo-Pacific *Heliacus (Teretropoma) infundibuliformis* (Gmelin, 1791) (Bieler, 1993: 260–264). NHMUK 1907.10.28.56, holotype (18.4 mm x 16.6 mm) (pursuant to ICZN Art. 74.6; if any additional syntypes are found, the holotype becomes the lectotype, deemed to have been designated by Bieler, and the additional syntypes become paralectotypes).

Omalogyridae

***rota, Skeneia** – Forbes & Hanley, 1850: 160–161, pl. 73, fig. 10, pl. 88, figs. 1, 2. Donegal, Warren & Barlee. Senior synonym of the type species of *Ammonicerina* O. G. Costa, 1861, *A. pulchella* O. G. Costa, 1861. The northeastern Atlantic *Ammonicerina rota* (Forbes & Hanley, 1850) (Sabelli et al., 1990: 220, as *Ammonicera* [a separate genus]; S. M. Smith & Heppell, 1991: 37); figured as *Ammonicera rota* by Fretter & Graham (1978b: 223–224, fig. 187). Type material not found in NHMUK or Leeds Museum in 2012.

nitidissima, Skeneia – “Forbes & Hanley”, 1850: 158–160, pl. 73, figs. 7, 8; 1853: 269. This was not intended to be a new species, as some authors have interpreted it (Sabelli et al., 1990: 220), but was rather an incorrect use of *Helix nitidissima* J. Adams, 1800. The latter has been interpreted as having been based on a juvenile land snail. The oldest name for the *Skeneia nitidissima* J. Adams, *sensu* Forbes & Hanley and other authors, is *Truncatella atomus* Philippi, 1841. Type species of the genus *Omalogyra* Jeffreys, 1859, by the subsequent designation of Tate (1868), essentially as *Helix nitidissima* J. Adams, *sensu* Forbes & Hanley (1850, 1853) (ICZN Code Art. Art. 70.3)

Pyramidellidae

***eulimoides, Odostomia** – 1844a: xxxvi; Hanley, 1844c: 18; Forbes & Hanley, 1850: 273–276; 1851: pl. 95, figs. 1–3. Guernsey (1844c); Herm near Guernsey (1844a), U.K. Attributed to W. Metcalfe in Sherborn (1926: 2232). *Brachystomia eulimoides* (Hanley, 1844) (Fretter et al., 1986: 601–603, figs. 414, 415; Sabelli et al., 1990: 224, as *Odostomia*; S. M. Smith & Heppell, 1991: 38). Type material not found in NHMUK or Leeds Museum in 2012.

rissoides, Odostomia – 1844a: xxxvi; Hanley, 1844c: 18; Forbes & Hanley, 1850: 284–287; 1851: pl. 96, figs. 4, 5; 1852: 279–280. Guernsey, U.K. Attributed to W. Metcalfe in Sherborn (1930: 5527). *Brachystomia rissoides* (Hanley, 1844) (Fretter et al., 1986: 599–601, figs. 412, 413); as a synonym of *B. scalaris* (Macgillivray, 1843) (Sabelli et al., 1990: 224; S. M. Smith & Heppell, 1991: 38). Aartsen (1987) discussed the valid-

ity of the name *scalaris* Macgillivray, which had been rejected by some authors when it was temporarily a junior homonym. NHMUK 1907.12.30.539–540 includes the specimen figured by Forbes & Hanley (1850), but no locality is associated with this lot. *Odostomia rissoides* Hanley, 1844, is the type species of *Brachystomia* Monterosato, 1884, by the subsequent designation of Crosse (1885), and of *Zastoma* Iredale, 1915, an unnecessary replacement name for *Brachystomia* Monterosato. Type material not found in NHMUK or Leeds Museum in 2012. USNM 132604, from the Jeffreys collection, is labeled as the “figured type,” but this is merely the specimen figured by Jeffreys in his *British conchology* (1869: pl. 73, fig. 4).

***striolata, Odostomia** – Forbes & Hanley, 1850: 267–268; pl. 95, fig. 5, ex Alder ms. Northumberland; Alder. This probably has nothing to do with the *O. striolata* listed by Jeffreys (1848: 339), ex Alder ms, as a *nomen nudum* in the synonymy of *O. turrita* Hanley. *Odostomia striolata* Forbes & Hanley, 1850 (Sabelli et al., 1990: 224; S. M. Smith & Heppell, 1991: 38). Type material not found in NHMUK or Leeds Museum in 2012.

***turrita, Odostomia** – Hanley, 1844a: [v], xxxvi–xxxvii, fig. 10; Hanley, 1844c: 18; Forbes & Hanley, 1850: 267, pl. 95, fig. 9, as a variety of *O. unidentata* (Montagu, 1803). Guernsey, U.K.; W. Metcalfe coll. Attributed to W. Metcalfe in Sherborn (1931: 6697). *Odostomia turrita* Hanley, 1844 (Fretter et al., 1986: 610–612, fig. 422; Sabelli et al., 1990: 224; S. M. Smith & Heppell, 1991: 38). Forbes & Hanley (1852: 279) later noted that their plate 94, fig. 8 (1850), which had also been labeled as *Odostomia unidentata* var. *turrita*, was instead an image of *O. dubia* Jeffreys, 1848; the latter seems now to be regarded as a synonym of *Brachystomia eulimoides* (Hanley, 1844). *Odostomia turrita* Hanley, 1844, is the type species of *Turritodostomia* Sacco, 1892, by original designation. Type material not found in NHMUK or Leeds Museum in 2012.

turrita, Parthenia – Hanley, 1844a: viii, xlv–xlv, fig. 91, ex Metcalfe ms; Jeffreys, 1848: 345; Forbes & Hanley, 1850: 305, the latter two as a synonym of *Odostomia excavata* Philippi, 1836. Guernsey, U.K. The latter is treated as *Folinella excavata* (Philippi, 1836) (Sabelli et al., 1990: 222; S. M. Smith & Heppell, 1991: 38); species figured in Fretter et al. (1986: 571–573, figs. 388, 389, as *Ividella excavata*). Type material not found in NHMUK or Leeds Museum in 2012.

jeffreysii, *Turbonilla* – “Forbes & Hanley, 1855”.

This combination has been cited by some authors, such as Fretter et al. (1986: 640), for *Melania scalaris* Philippi, 1836, *non M. scalaris* Spix & Wagner, 1827. However, this name was not first proposed by Forbes & Hanley. Instead, it first appeared as a *nomen nudum* in Jeffreys (1842: 237) as *Pyramidella jeffreysii*, ex Clark ms, in the synonymy of *Eulima decussata*, also a *nomen nudum*. It next appeared in Jeffreys (1848: 346) as *Turbonilla (Pyrgiscus) jeffreysii*, ex Clark ms, in the synonymy of *Odostomia scalaris* (Philippi, 1836). It then appeared in Forbes & Hanley (1850: 251): “Among collectors this shell has generally been distinguished by the manuscript name *jeffreysii*”. The first person to have treated the name as available before 1961 (ICZN Code, 1999: Art. 11.6.1) appears to be Winckworth (1932: 227), who cited it as of Forbes & Hanley, 1850, along with mention of *scalaris* and citation of the relevant pages from Forbes & Hanley (1850) and Jeffreys (1867). It was not listed by Warén (1980) as a Jeffreys name. However, according to this provision of the Code, it should be dated from Jeffreys (1848), where it first appeared in the synonymy of an available taxon. It is thus treated by recent authors, such as Aartsen (1987), Sabelli et al. (1990: 226) and S. M. Smith & Heppell (1991: 40, 84).

Rissoellidae

****globularis*, *Jeffreysia*** – Forbes & Hanley, 1852: 268–269, pl. 133, fig. 5, ex Jeffreys ms. Skye and the Shetlands; Barlee. *Rissoella (Jeffreysina) globularis* (Forbes & Hanley, 1852) (Fretter & Graham, 1978b: 220–221, fig. 184, as “Jeffreys”; Sabelli et al., 1990: 219; S. M. Smith & Heppell, 1991: 37). *Jeffreysia globularis* Forbes & Hanley, 1852, is the type species of *Jeffreysina* Thiele, 1925, by the subsequent designation of Wenz (1939). Type material not found in NHMUK or Leeds Museum in 2012. USNM 185002, from the Jeffreys collection, is labeled as the “figured type,” but this is merely the specimen figured by Jeffreys in his *British conchology* (1869: pl. 69, fig. 7).

Limacinidae

jeffreysii*, *Spirialis – Forbes & Hanley, 1849: 386, pl. 57, fig. 8. Shores of the English

Channel, Jeffreys. Synonym of *Limacina retroversa* (Fleming, 1823) (Sabelli et al., 1990: 238–239). Type material not found in NHMUK or Leeds Museum in 2012. USNM 169369, from the Jeffreys collection, is labeled as “type,” but it is from Tenby (Wales), which is not on the English Channel, so it is not from the type locality.

macandrewi*, *Spirialis – Forbes & Hanley, 1849: 385–386, pl. 57, figs. 6, 7. Dredged in 60 fms. [110 m], 15 miles south of Mizen Head, south of Ireland, M'Andrew, June 1848. Synonym of *Limacina retroversa* (Fleming, 1823) (Sabelli et al., 1990: 238–239). Type material not found in NHMUK or Leeds Museum in 2012. USNM 169364, from the Jeffreys collection, is labeled as the “figured type,” but this is merely the specimen figured by Jeffreys in his *British conchology* (1869: pl. 98, fig. 5).

Placobranchidae

Elysiidae – Forbes & Hanley, 1851: 613, as Elysiadae. Regarded as a synonym of Placobranchidae Gray, 1840 (Bouchet & Rocroi, 2005: 260).

Lomanotidae

Lomonotus “Forbes & Hanley”, 1851: 585. This is a misspelling of *Lomanotus* Vérany, 1844, as noted by Neave (1939, vol. 2: 987).

Flabellinidae

Flabellina “Forbes & Hanley”, 1851: 592. This was so listed by Neave (1939, vol. 2: 410), but this genus was first made available by Voigt (1834) (ICZN Opinion 781, 1966).

Eubranchidae

Cavolina – Forbes & Hanley, 1851: 297, as “Section III” of *Eolis*. Type species (SD Lemche, 1969, Note within Opinion 883): *Eubranchus tricolor* Forbes, 1838. Junior objective synonym of *Eubranchus* Forbes, 1838 (discussed in ICZN Opinion 883, 1969).

Retusidae

****carpenteri*, *Bulla (Cylichna)*** – Hanley, 1859a: 543–544. Mazatlán [Sinaloa], Mexico; Hanley coll. *Sulcoretusa carpenteri* (Hanley, 1859) (Keen, 1971: 796–797, holotype figured). NHMUK 1907.12.30.249, holotype.

Siphonariidae

***belcheri**, *Siphonaria* – Hanley, 1858c: 151, 153. Probably “Indian Seas”; E. Belcher; Hanley coll. *Siphonaria belcheri* Hanley, 1858, from the Persian Gulf (Hubendick, 1946: 34, 90, pl. 2, figs. 5–8); recently established in the eastern Mediterranean Sea (Albayrak & Caglar, 2006). NHMUK 1900.3.19.28–34, seven syntypes.

blainvillei, *Siphonaria* – Hanley, 1858c: 151, 153. Loc. unknown; Hanley coll. Synonym of *S. funiculata* Reeve, 1856, from Tasmania (Hubendick, 1946: 23, 89, pl. 1, figs. 15–17; Jenkins, 1981: 2, pl. 1a). NHMUK 1907.10.28.90, holotype (13.6 mm x 17.9 mm).

brunnea, *Siphonaria* – Hanley, 1858a: 24; 1858c: 151. Bermuda; Cuming coll. Synonym of *S. alternata* (Say, 1826) from Florida and the Caribbean (Hubendick, 1946: 44–45, 90, pl. 3, figs. 1–6). NHMUK 19818, four syntypes.

***carbo**, *Siphonaria* – Hanley, 1858a: 24; 1858c: 151. Loc. unknown; Cuming coll. *Siphonaria carbo* Hanley, 1858 (Hubendick, 1946: 35, 93, pl. 6, figs. 16, 17). Hubendick (1946) reported this species as coming from Natal (South Africa); Chambers & McQuaid (1994) incorrectly stated that this species “was described from the Caribbean,” so that “we therefore consider that *S. carbo* does not occur on South African shores and adopt *S. nigerrima* Smith, 1903 as the correct name for the South African species.” That conclusion was incorrect since Hanley did *not* have any type locality for this species. NHMUK 19819, holotype (7.6 mm x 21.3 mm).

exulum, *Siphonaria* – Hanley, 1858a: 25; 1858c: 152, as “*exulorum*”. Norfolk Island; Hanley coll. Synonym of *S. diemenensis* Quoy & Gaimard, 1833, which occurs in New Zealand, Australia and Norfolk Island (Hubendick, 1946: 38–39, 90, pl. 2, figs. 14–17). NHMUK 1900.3.19.27, holotype (5.9 mm x 14.1 mm).

***nutallii**, *Siphonaria* – Hanley, 1858c: 152, 153. Hawaii; Frick; Cuming coll. *Siphonaria nutallii* Hanley, 1858, of Hawaii (Hubendick, 1946: 51–52, 91, pl. 4, figs. 1–4, as “*nutalli*”); species not mentioned by Kay (1979: 493) or Severns (2011). NHMUK 20120166, five syntypes; Leeds Museum 1957.173.612, seven possible syntypes (“Sandwich Isles”).

parma, *Siphonaria* – Hanley, 1858a: 24–25; 1858c: 152. West Africa; Cuming coll. Synonym of *S. pectinata* (Linnaeus, 1758), which occurs in both the eastern and western Atlan-

tic (Hubendick, 1946: 31–33, 89, pl. 1, figs. 30–39; Kawauchi & Giribet, 2011). NHMUK 198110, holotype (6.1 mm x 17.6 mm).

redimiculum, *Siphonaria* – “Hanley” – Listed by Norris & Dance (2002: 373) as “var.” and “Proc. zool. Soc. London 25: 25”. However, this species was actually described by Reeve, 1846 (pl. 5, fig. 24). Reeve’s species has been listed as a synonym of *S. lateralis* Gould, 1846 (p. 153), from Patagonia, Falkland Islands, and elsewhere in the sub-Antarctic (Hubendick, 1946: 26–27, 89, pl. 1, figs. 22–25, as *Kerguelenia*), more recently been placed into *Kerguelenella* (Powell, 1979: 293). However, Reeve’s species dates from March 1846, whereas Gould’s species was published in August, so if they are regarded as synonyms, Reeve’s name would take precedence.

rumphii, *Siphonaria* – “Hanley” – Listed by Norris & Dance (2002: 373) as “Proc. zool. Soc. Lond. 26: 153” but not present in Hanley (1858a) and not listed by Hubendick (1946). Possibly a transcription error by Norris & Dance involving *Siphonaria nutallii*, missing from their list, and *Pinna rumphii*.

Lymnaeidae

strigata, *Limnaea ovalis* var. – Hanley & Theobald, 1873: 30, pl. 70, fig. 4. Jounpore. *Non Limnaea pinguis* var. *strigata* G. B. Sowerby II, 1872 (= *Catascopium catascopeum* (Say, 1867)). Most likely a synonym of *Radix luteola* (Lamarck, 1822), because *ovalis* is a synonym of *luteola* (Brandt, 1974: 232–233, 376, pl. 16, fig. 98). Starmühlner (1974: 162) listed this as a junior synonym of *Radix (Cerasina) luteola* var. *pinguis* (Dohrn, 1858). Ramakrishna & Dey (2007: 208–209) listed *Lymnaea (Pseudosuccinea) luteola* Lamarck, 1822, but did not mention Hanley & Theobald’s taxon. Type material not found in NHMUK or Leeds Museum in 2012.

sylhetica, *Limnaea ovalis* var. – Hanley & Theobald, 1873: 30, pl. 70, fig. 9. Marshes in Sylhet. Listed in Preston, 1915: 109. Most likely a synonym of *Radix luteola* (Lamarck, 1822), because *ovalis* is a synonym of *luteola* (Brandt, 1974: 232–233, 376, pl. 16, fig. 98). Ramakrishna & Dey (2007: 208–209) listed *Lymnaea (Pseudosuccinea) luteola* Lamarck, 1822, but did not mention Hanley & Theobald’s taxon. Type material not found in NHMUK or Leeds Museum in 2012.

Planorbidae

coromandelianus, *Planorbis* – Hanley, 1859c: 239, as “Fabric. in Dohrn’s MS”, *nomen nudum*.

Legrandia – Hanley in Legrand, 1872: 27, *nomen nudum*; *non Legrandia* Beddome, 1883. This generic name and the species name below [*maddocki*] were mentioned in a letter from Hanley quoted by Legrand, evidently pleased to be honored with a genus of freshwater limpet. Hanley said, “A description with figures will appear in my monograph of Tasmanian freshwater shells now in course of preparation.” No such monograph ever appeared. Legrand (1879: 95) later noted that the species involved, “the finest ... of the genus”, had been named as *Ancylus cumingianus* Bourguignat, 1854 (pp. 91–92, pl. 25, figs. 1–9). The generic name *Legrandia* was made available by Beddome (1883: 91) for a marine fissurellid gastropod, also from Tasmania. Ignoring that the name was nude, Iredale (1943: 230) treated the freshwater limpet genus as being valid. The freshwater limpet has been listed as a synonym of *Pettancylus* Iredale, 1943, or of *Ferrissia* Waker, 1903; B. J. Smith (1992: 266) concluded that *Legrandia* was a junior synonym of *Ancylastrum* Bourguignat, 1854.

maddocki, Legrandia – Hanley, in Legrand, 1872: 27, *nomen nudum*. Sometimes listed as a synonym of *Ferrissia* (*Pettancylus*) *petterdi* (Johnston, 1879) or of *Ferrissia* (*Pettancylus*) *tasmanicus* (Tenison-Woods, 1880), two *Ferrissia* taxa described from Tasmania. B. J. Smith (1992: 266) concluded that this species was a junior synonym of *Ancylastrum cumingianus* (Bourguignat, 1854), and stated that Hanley’s type material was “presumed lost”, illogical because a *nomen nudum* cannot have type material.

merguiensis, Planorbis – Hanley & Theobald, 1876: 60, pl. 151, figs. 5, 6. Mergui, Birmah, ex Philippi ms. Synonym of the southeast Asian *Indoplanorbis exustus* (Deshayes, 1834) (Brandt, 1974: 234–235, 376, pl. 16, fig. 99). Type material not found in NHMUK or Leeds Museum in 2012.

Onchidiidae

celticum, Onchidium – “Forbes & Hanley, 1852”. This was so listed by Fretter (1943) and by Norris & Dance (2002: 370), but this name was actually first made available by Cuvier, in Audouin & Milne-Edwards, 1832.

Now known as *Onchidella celtica* (Cuvier, 1832) (Dayrat, 2009: 4, 10).

Succineidae

****collina, Succinea*** – Hanley & Theobald, 1873: 30, pl. 68, figs. 8, 9, ex Blanford ms; Blanford, 1881: 200–201. Mahabaleshwar. *Succinea collina* Hanley & Theobald, 1873 (Gude, 1914: 449; Ramakrishna et al., 2010: 209–210). NHMUK 1907.12.30.224–225, two syntypes (from Blanford collection).

Valloniidae

****evezardi, Pupa*** – Hanley & Theobald, 1874: 41, pl. 101, figs. 5, 6, ex Blanford ms; Blanford, 1881: 199–200, as *Pupa* (*Pupisoma*). Singhur Hill, Dekkan. *Pupisoma evezardi* (Hanley & Theobald, 1874) (Gude, 1914: 37–38, as “Blanford”; Ramakrishna et al., 2010: 124, also as “Blanford”). NHMUK 1923.06.01 may be a specimen subsequently collected by Blanford, from Khandala.

Clausiliidae

****bacillum, Clausilia*** – Hanley & Theobald, 1870: 12, pl. 24, fig. 1, ex Benson ms. Nanclai, Khasi Hills. *Euphaedusa bacillum* (Hanley & Theobald, 1870) (Mittra et al., 2005: 141–142; Ramakrishna et al., 2010: 141); as *Cylindrophaedusa* (*Montiphaedusa*) *bacillum* (Hanley & Theobald, 1870) (Nordsieck, 2002: 87; 2007: 26). Type material not found in NHMUK or Leeds Museum in 2012.

****tuba, Clausilia*** – Hanley, 1868b: 343; Hanley & Theobald, 1870: 12, pl. 24, fig. 9. Shan Province, India; Hanley & Theobald colls. *Indonenia tuba* (Hanley, 1868) (Nordsieck, 2007: 37). NHMUK 1888.12.4.1033, syntype; NHMUK 1904.12.30.241, figured syntype (33.4 mm x 5.7 mm).

Subulinidae

Note: the taxa described by Hanley in the “Achatinidae” are now all referred to the Subulinidae.

****bottampotana, Achatina*** – Hanley & Theobald, 1876: 63, pl. 156, fig. 1, ex Beddome ms. No locality provided. *Glessula bottampotana* (Hanley & Theobald, 1876) (Gude, 1914: 401; Ramakrishna et al., 2010: 154). Both references used the unjustified emendation “*bol-lampattiana*”, because the species was supposed to be from the Bollampatty Hill. However,

because this cannot be ascertained from the original description, this must be considered an unjustified emendation (ICZN Code Art. 33.2). Type material not found in NHMUK or Leeds Museum in 2012.

illustris, *Achatina (Electra) – Hanley & Theobald, 1874: 41, pl. 102, fig. 9, ex Godwin-Austen ms; Godwin-Austen, 1875: 3, pl. 1, fig. 5. Cachar. *Glessula illustris* (Hanley & Theobald, 1874) (Gude, 1914: 421–422; Ramakrishna et al., 2010: 162, both with “Godwin-Austen” as author). NHMUK 1985158, four possible syntypes from Godwin-Austen’s collection (Hengdan Peak, North Cachar Hills, India); since Hanley & Theobald published Godwin-Austen’s name, it is possible that they relied upon Godwin-Austen’s specimens.

isis, *Achatina (Glessula) – Hanley, 1876: 606; Hanley & Theobald, 1876: 62, pl. 155, fig. 5. India meridionalem; Hanley coll. *Glessula isis* (Hanley, 1876) (Gude, 1914: 393–394; Ramakrishna et al., 2010: 163). Type material not found in NHMUK or Leeds Museum in 2012.

sattaraensis, *Achatina (Electra) – Hanley & Theobald, 1873: 33, pl. 78, fig. 4, ex H. Adams ms. Sattara, Bombay, India. Not an Adams species (and thus not listed in Trew, 1992), but rather a new name for *Glessula fusca* H. Adams, 1868, *non Achatina fusca* Pfeiffer, 1852. *Glessula sattaraensis* (Hanley & Theobald, 1873) (Gude, 1914: 432; Ramakrishna et al., 2010: 173, the latter as the unjustified emendation “sattarensis”). Theobald (1876: 52a) said: “This shell, it may be presumed, came from Western India rather than either Saharunpur or Ceylon,” as incorrectly cited by Hanley & Theobald. NHMUK 1986032 is labeled the “holotype”, but this was Hanley & Theobald’s specimen, so it cannot be the type of this renamed homonym (ICZN Code Art. 72.7). NHMUK 1878.1.28.632 is the true holotype of H. Adams (and Hanley & Theobald), but unfortunately it was “crushed in the box” according to the label and no longer extant as of 2012.

senator, *Achatina (Glessula) – Hanley, 1876: 606; Hanley & Theobald, 1876: 62, pl. 155, fig. 4. Cottyam Hills, India meridionali; M’Andrew & Beddome colls. *Glessula senator* (Hanley, 1876) (Gude, 1914: 393; Ramakrishna et al., 2010: 174). Type material not found in NHMUK or Leeds Museum in 2012.

theobaldi, *Achatina (Electra) – Hanley & Theobald, 1870: 9, pl. 17, fig. 5. “Near the Salwen.” *Bacillum theobaldi* (Hanley & Theobald, 1870) (Gude, 1914: 344–345; Ramakrishna

et al., 2010: 150, both incorrectly with only Hanley as author). Type material not found in NHMUK or Leeds Museum in 2012.

Bacillum – Theobald in Hanley & Theobald, 1870: 17. Proposed as a subgenus of *Achatina*. “The sub-genus *Bacillum* is proposed by Mr. Theobald for this [*Achatina obtusa* Blanford, 1869 (Burma)], the preceding [*A. cassiaca* Reeve, ex Benson ms. (Khasia Hills)], and other allied forms.” Type species (subsequent designation of Pilsbry, 1906: 1): *Achatina cassiaca* Reeve, 1849. Used as a valid genus without discussion (Ramakrishna et al., 2010: 148). Not a Hanley taxon, because it was expressly credited to Theobald in the Hanley & Theobald work.

lyrata, Achatina (Electra) – Blanford, 1870: 20–21 [March]; Hanley & Theobald, 1870: pl. 18, fig. 9, ex Blanford ms. This species has to be credited to Blanford, who published it at a known date in 1870, whereas its appearance in Hanley & Theobald (1870) has to be dated at the end of the year. *Glessula lyrata* (Blanford, 1870) (Gude, 1914: 441–442); not mentioned by Ramakrishna et al. (2010: 165).

Corillidae

beddomeae, *Helix (Plectopylis) – Hanley & Theobald, 1876: 60, pl. 150, figs. 1–2. “Ceylon or Southern India.” [Named after the wife of Colonel Beddome]. *Corilla beddomeae* (Hanley & Theobald, 1876) (Satyamurti, 1960: 92–93; Naggs & Raheem, 2000: viii, 113, fig. 139). Type material not found in NHMUK or Leeds Museum in 2012.

Parmacellidae

tennentii, *Parmacella – Hanley, 1859c: 238, ex Templeton ms. Kalany Ganga, near Ruanwellé, Ceylon [Sri Lanka]. Type material not found in NHMUK or Leeds Museum in 2012.

Ariophantidae

ingrami, *Helix – Hanley & Theobald, 1872: 27, pl. 60, figs. 9–10, ex Blanford ms; Blanford, 1881: 193–195, as “*Sesara? ingrami*”. Aracan Hills (Benson coll.). *Sesara ingrami* (Hanley & Theobald, 1872) (Blanford & Godwin-Austen, 1908: 248). Type material not found in NHMUK or Leeds Museum in 2012.

phidias, *Helix – Hanley & Theobald, 1876: 59, pl. 149, fig. 4, ex Thorp ms. Upper Ouvah, Ceylon; F. Layard. *Euplecta phidias* (Hanley & Theobald, 1876) (Blanford & Godwin-Austen, 1908: 71; Naggs & Raheem, 2000: v, 49, fig. 63). Type material not found in NHMUK or Leeds Museum in 2012.

reevei, *Helix juliana* – Hanley & Theobald, 1872: 24, pl. 52, fig. 7. Ceylon. Possible synonym of *Ariophanta (Cryptozona) juliana* (Gray, 1834). Type material not found in NHMUK or Leeds Museum in 2012.

solida, *Helix semidecussata* var. – Hanley & Theobald, 1872: 27, pl. 58, fig. 2. Ceylon. Synonym of *Euplecta semidecussata* (L. Pfeiffer, 1853) (Blanford & Godwin-Austen, 1908: 57–58). Type material not found in NHMUK or Leeds Museum in 2012.

vidua, *Helix – Hanley & Theobald, 1875: 52, pl. 130, figs. 2, 3, ex Blanford ms; Blanford, 1881: 190–191, 222, pl. 2, fig. 5. Khasi Hills. Type species of the genus *Khasiella* Godwin-Austen, 1899 (pp. 129–132), by the subsequent designation of Blanford & Godwin-Austen (1908: 157). *Khasiella vidua* (Hanley & Theobald, 1875) (Blanford & Godwin-Austen, 1908: 158–159; Mitra et al., 2005: 272–273; Ramakrishna et al., 2010: 299–300). NHMUK 1906.2.2.307, possible syntype.

Bradybaenidae

arakanensis, *Helix tapeina var. – Hanley & Theobald, 1870: 8, pl. 15, fig. 10, ex Theobald ms; Hanley & Theobald, 1876: vii, as a full species of *Helix*. Assam. *Plectotropis arakanensis* (Hanley & Theobald, 1870) (Gude, 1914: 503, who credited the species solely to Theobald). Type material not found in NHMUK or Leeds Museum in 2012.

concolor, *Helix zoroaster* var. – Hanley & Theobald, 1874: 36, pl. 86, figs. 2, 3. Near the Irawadi, between Prome and Ava. Synonym of *Bradybaena zoroaster* (Theobald, 1859) (Gude, 1914: 202–203, as *Eulota*). Now placed in *Bradybaena* (R. Bank, pers. comm., 29 Jan. 2012). Type material not found in NHMUK or Leeds Museum in 2012.

Camaenidae

andamanicus, *Bulimus – Hanley & Theobald, 1876: 59, pl. 148, fig. 10, ex Thorp ms. Andaman Islands. *Amphidromus andamanicus* (Hanley & Theobald, 1876) (Gude, 1914: 184–185; Subba Rao & Mitra, 1991: 69, pl. 5, as a subspecies of *A. furcillatus* in text only; Mitra et al., 2005: 299–300; Ramakrishna et al., 2010:

322 (as a full species). Type material not found in NHMUK or Leeds Museum in 2012.

anserina, *Helix* – “Hanley & Theobald”, 1874: 46, pl. 112, figs. 7, 10. Unjustified emendation of *Helix ansorinus* Theobald, 1866; also used by Theobald (1876: 26) and Gude (1914: 175–176). Now *Chloritis ansorina* (Theobald, 1866).

Unknown Pulmonata

nietneri, *Helix* – Hanley, 1859c: 239, ex Dohrn ms, *nomen nudum*. Ceylon [Sri Lanka].

CEPHALOPODA

umbilicatus, *Nautilus* – “Hanley”, 1840 & 1842a: viii, 145, text fig. Listed by Tomlin (1945: 91) and by Norris & Dance (2002: 374) as a Hanley species, this was more likely simply a misspelling of *N. umbilicatus* Linnaeus, 1758, so there would be no “Hanley” type material.

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Vol.	Part	Pages	Plates	Date
1	1	1–40	A, B, 1, 2	Jan. 1, 1848
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	51	479–486 title page, vii–lxxx		May 1, 1853
2	51	title page, v–viii		May 1, 1853
	13	1–40	P, 35–37	Jan. 1, 1849
	14	41–80	R, 38–40	Feb. 1, 1849
	15	81–120	Q, 41, 43, 44	March 1, 1849
	16	121–160	S, 45–47	April 2, 1849
	17	161–200	U, 42, 48, 49	May 1, 1849
	18	201–240	50–53	June 1, 1849
	19	241–280	AA, 54–56	July 2, 1849
	20	281–320*	BB, 58, 59, 61	Aug. 1, 1849
	21	321–360	NN, 57, 62, 63	Sept. 1, 1849
	22	361–400	DD, 64–66	Oct. 1, 1849
	23	401–440	FF, 60, 67, 68	Nov. 1, 1849
	24	441–480	II, 69–71	Dec. 1, 1849
	25	481–520	CC, 72, 73, 77	Jan. 1, 1850
	26	521–557	T, 74, 78, 79	Feb. 1, 1850
3	51	title page, v–x		May 1, 1853
	27	1–40	EE, 83–85	March 1, 1850
	28	41–80	GG, HH, 80, 86	April 1, 1850
	29	81–120	JJ, 75, 76, 81	May 1, 1850
	30	121–160	LL, 82, 87, 115	June 1, 1850
	31	161–200	KK, 88, 116, 117	July 1, 1850
	32	201–240	OO, 118–120	Aug. 1, 1850
	33	241–280	PP, 89, 92, 121	Nov. 1, 1850
	34	281–320	“SS” [= XX], 90, 91, 93	Dec. 2, 1850
	35	321–360	RR, 94–96	Jan. 1, 1851
	36	361–400	SS, 97, 99, 100	Feb. 1, 1851
	37	401–440	TT, 101–103	March 1, 1851
	38	441–480	UU, 104–106	April 1, 1851
	39	481–520	VV, 107–109	May 1, 1851
	40	521–560	110–113	June 2, 1851
	41–42	561–616	YY, ZZ, 98, 114, 114A–D	Sept. 1, 1851
4	51	title page, iii–vi		May 1, 1853
	43	1–40	DDD, 114E, 114F, 123	Jan 1, 1852
	44	41–80	“FFF” [= GGG], 124–126	Feb. 2, 1852
	45	81–120	EEE, 129–131	March 1, 1852
	46	121–160	FFF*, 122, 127, 128	April 1, 1852
	47	161–200	HHH, KKK, LLL, MMM	July 1, 1852
	48	201–240	BBB, OOO, PPP, QQQ	Sept. 1, 1852
	49	241–280	AAA, CCC, 132, 133	Dec. 1, 1852
	50	281–302	W, MM, III, JJJ, NNN, RRR, SSS	May 1, 1853

Notes: Part numbers were on part covers and are not evident in bound volumes. Fisher & Tomlin (1935) listed Parts 50 and 51 as being issued together. It can be assumed that Part 51 contained the end pages for Vol. 1 (Explanation of the Plates of Animals, pp. 479–486) as well as the Title page, Dedication, Preface (vii–ix), General Index (xxxvii–lxxv), and Contents (lxxvii–lxxx). It also contained the title contents pages for Volumes 2–4 (Woodward, 1901). The verso of page 301 in Vol. 4 is unnumbered but is imprinted with instructions to binders and is therefore considered an included page. The numbered plates are of empty shells, while the lettered plates show live animals.

In Part 34, Plate XX was incorrectly numbered as SS in a few copies of the work – the misnumbered plate depicts *Pleurobranchus*; the true Plate SS depicts *Fusus*. In Part 44, Plate GGG was misnumbered as FFF. This plate depicts *Helix* and other snails. To avoid confusion, what should have been FFF was issued as FFF* (depicting *Arion* and other slugs). The plates are dated to year. There are no plates numbered J, V, X, Z, QQ, or WW. Sometimes listed as having 210 plates, without noting the omissions, and miscataloged in many libraries as having 197 plates, with plates 114A–F being overlooked. [Note that pages 309–320 of volume 3(20) were reprinted in the *Edinburgh New Philosophical Journal* 47(94): 239–248, Oct. 1849, erroneously credited to “Forbes & Stanley [sic]”; this excerpt was listed by Norris & Dance (2002: 376) as if it were a separate article by Hanley & Forbes.].

It should be noted that Damon (1857), a shell dealer, published an 11-page checklist of the names in Forbes & Hanley, and this is catalogued in at least one library as if it were by Forbes & Hanley, the title page having been lost.

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Part	Pages	Plates	Date
1	1–10	1–20	1870
2	11–18	21–40	1870
3	19–28	41–60	1872
4	29–34	61–80	1873
5	35–40	81–100	1874
6	41–48	101–120	1874
7	49–56	121–140	1875
8	57–65, i–xviii	141–160	1876

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i–viii + 1–83 [84 blank]	1–95	Bruguière	30 July 1791
85–132	96–189, 107a–c	Bruguière	May 1792
	190–286	Bruguière	8 July 1797
	287–390	Lamarck	29 April 1798
1–16	391–488, 431bis, 431bis*	Lamarck	14 Dec. 1816
83 [repr.]–84, 133–180		Bory de Saint-Vincent	27 Sept. 1827

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Pages	Plates	Date	Lief.
1–16	1–6	1910	545
17–40	7–12	1910	546
41–64	12a, 13–17	1911	549
65–88	18–23	1911	551
89–112	24–29	1911	554
113–136	30–35	1912	559
137–160	36–41	1913	566
161–184	42–47	1913	569
185–208	48–53	1914	571
209–256	54–59	1914	573
257–288	60–63	1919	581
289–304	64–68	1920	582
305–344	69–73	1920	584

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RESEARCH NOTES

A CASE OF PHORESIS OF SPHAERIIDS BY CORIXIDS: FIRST REPORT FOR THE AMERICAS

Diego G. Zelaya^{1,2*} & María Cristina Marinone¹

INTRODUCTION

Dispersal represents a key process in determining the genetic structure and demography of populations. This process reduces competition among siblings, reduces competition between parents and offspring, reduces the likelihood of inbreeding, increases recolonization following local extinctions, also decreasing the risk of local extinction (Pechenik, 1999). Similarly, the modes of reproduction and dispersal can play a major role in species longevity, geographic distribution, and rate of speciation (Mayr, 1970; Stanley, 1979).

In freshwater bivalves, a free-living larval stage is frequently suppressed; consequently, the dispersal of a species relies on the capacities of juvenile and adult stages. Dispersal mechanisms include rafting (i.e., the transport of an organism on a drifting object); current-mediated dispersal of juveniles suspended in water (McKillop & Harrison, 1982); airborne dispersal (anemochory) of juveniles and adults (e.g., hurricanes, tornadoes, and other twist-ers) (Rees, 1965, and references therein); and dispersal by biotic (either natural or anthropogenic) vectors, with vertebrates or invertebrates acting as carriers (Kappes & Haase, 2012, and references therein).

Darwin (1882) was the first to assess the evolutionary importance of the long-distance transportation of freshwater molluscs by insects. The present paper provides the first record on the occurrence of this phenomenon in the Americas.

MATERIAL AND METHODS

Bivalves associated to insects were found in a small (550 m²) artificial reservoir located at 16°59'33.43"S, 68°04'30.83"W, approximately 90 km southeast of Lake Titicaca Menor (or Lake Huiñaimarca), Bolivia, at 3,993 m altitude.

This temporary water body was very shallow (< 0.6 m), turbid (clayey), with sandy-muddy bottom, devoid of aquatic vegetation, and used as a cattle drinking trough (Fig. 1).

The climate of this region is characterized by reduced atmospheric humidity, high levels of UV radiation, low temperatures (extreme daily temperatures ranging from -10 to 21°C), marked daily fluctuations of temperature (up to 25°C) due strong solar radiation and intense irradiative cooling by night. Scarce rainfalls (< 300 mm/year) are concentrated during the austral summer.

Qualitative invertebrate samples were taken with a hand net (320 µm mesh). Since the sampling method was not intended for infaunal organisms, molluscs were collected unexpectedly because of their attachment to nectonic organisms. Samples were preserved in situ in ethanol. All the specimens were measured with a precision of 0.025 mm with a Leica M80 stereoscopic microscope. A preliminar identification of bivalves was made based on shell morphology. In the absence of adult specimens, their identity was confirmed by Dr. Taehwan Lee, by using molecular information from 16S, COI and ITS1 gene sequences. Voucher specimens were deposited in the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN-In 38868), Argentina and in the University of Michigan, USA.

RESULTS

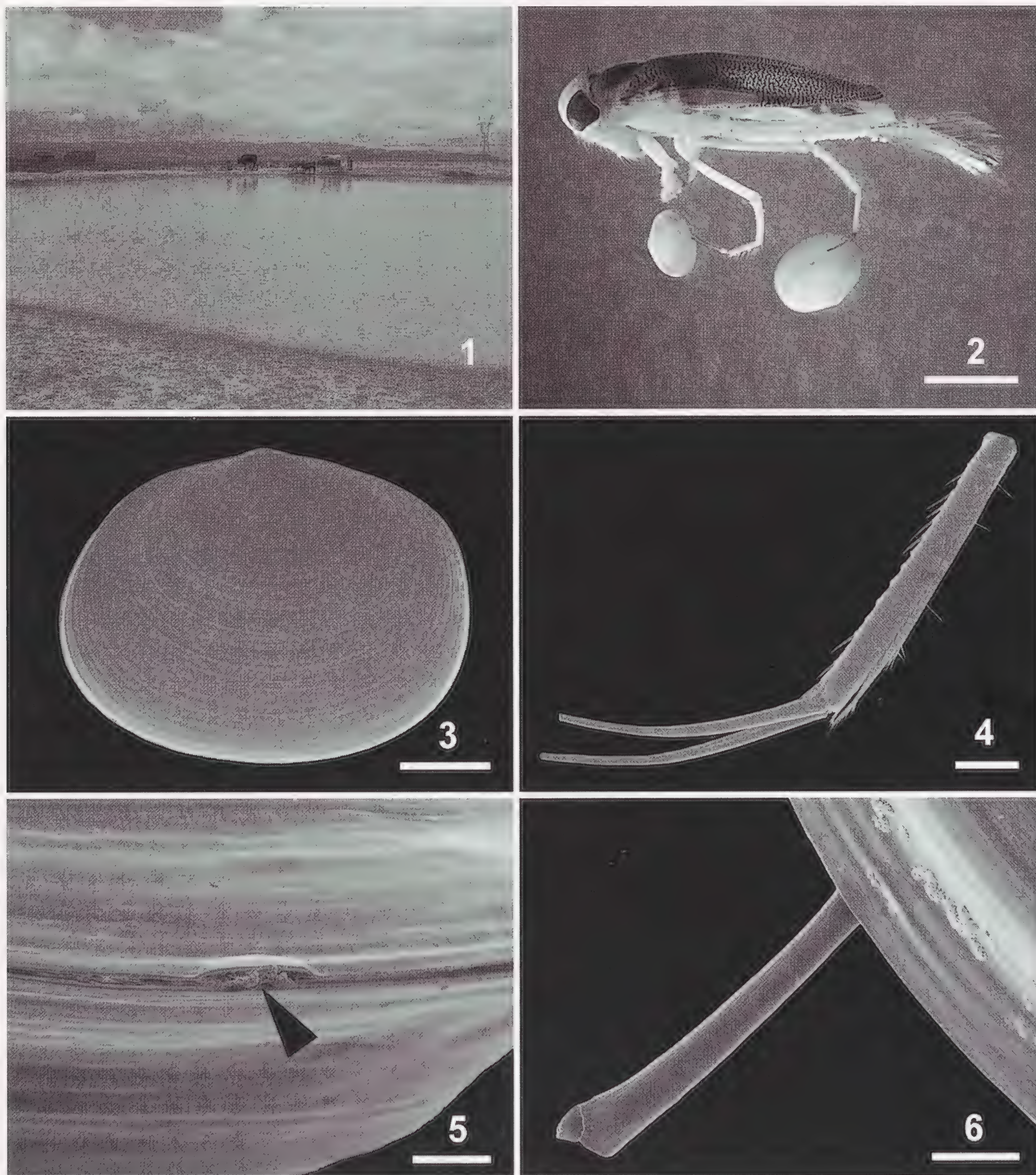
Thirty-four juvenile (1.50–2.45 mm length) fingernail clams were collected. The shell shape of the examined juvenile specimens (Fig. 3) agrees with the description provided by Kuiper & Hinz (1984) for *Sphaerium* (*Musculium*) *auricochae* (Philippi, 1869) (Veneroida: Sphaeriidae).

Seven specimens of *Ectemnostega* (*Ectemnostegella*) *quechua* Bachmann, 1961

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FIGS. 1–6. Phoresis of sphaeriids by corixids. FIG. 1: Collecting site; FIG. 2: *Ectemnostega quechua* carrying two juveniles of *Sphaerium lauricochae* clipped to the tarsal claws of its middle legs. Scale bar = 2 mm; FIG. 3: Outer left view of a juvenile specimen of *S. lauricochae*. Scale bar = 500 μ m; FIG. 4: Tips of the tarsal claws of a middle leg of *E. quechua* damaged by the attachment and detachment of a juvenile of *S. lauricochae*; FIG. 5: Ventral view of a juvenile of *S. lauricochae*. The arrow shows the injury produced in the shell margin after the attachment to a corixid claw. Scale bar = 100 μ m; FIG. 6: Lateral view of a juvenile of *S. lauricochae* with a claw fragment trapped within the valves. Scale bar = 100 μ m.

(Heteropta: Corixidae) were found carrying fingernail clams (Fig. 2). From them, 6 were adult specimens, both brachypterous (male: 7.1 mm long; females: 7.2, 7.3, 7.5 mm long) and macropterous (females: 7.9, 8.3 mm long), and a nymph of the 5th instar (5.6 mm long).

The phoretic relationship of *S. lauricochae* with *E. quechua* was verified in only one out of 42 water bodies surveyed. In all cases, the clams were clipped either to one or both tarsal claws of the middle legs. Usually a single bivalve per corixid was found, except for one that carried two of them (Fig. 2). In the latter case, one bivalve on each side of the insect was found (Fig. 2). The tight attachment of fingernail clams to corixids damage both the shell margin (it may show an indentation or a claw fragment within shell: Figs. 5, 6) and the insect claws (they present trimmed tips: Fig. 4). Therefore, direct and indirect evidences show that at least 44% of the clams collected in the finding place were involved in the association. The attachment to insects occurs over the whole size range of the studied bivalves.

DISCUSSION

Sphaeriids are brooding bivalves that retain their embryos in marsupial pouches derived from parental gills (Heard, 1977). Known dispersal mechanisms of Sphaeriidae include longitudinal movements in flowing waters, and lateral movements among unconnected habitats by biotic and abiotic vectors. In this regard, fishes and birds have been reported as carriers of sphaeriids that, after being ingested, can survive gut passage (Brown, 2007); other animals have been reported carrying sphaeriids attached to their body (Mackie, 1979). The latter condition is known for bivalves clamped onto ducks (Green & Figuerola, 2005), salamanders (Davis & Gilhen, 1982; Wood et al., 2008), frogs (Gutleb et al., 2000), and insects (Rees, 1965; Mackie, 1979). Except for some reports on bivalves clipped to Odonata and Coleoptera (Kew, 1893), most of them involve hemipterans of the genera *Nepa*, *Notonecta*, *Corixa*, and *Sigara* (Kew, 1893; Cash, 1912; Griffith, 1945; Fernando, 1954; Lansbury, 1955; Soldán et al., 1989). Furthermore, except for the report by Griffith (1945) on "small clams" carried by nymphs of Corixidae, all other references involve adult insects as carriers.

The present study provides the first record for the Americas of fingernail clams transported by insects. The sphaeriid *Sphaerium lauricochae* has been reported from lentic and lotic freshwater habitats of the Altiplano of Bolivia, Chile, Peru, and northwestern Argentina (Kuiper & Hinz, 1984; Ituarte, 2005; Parada & Peredo, 2006). On the other hand, the heteropteran *Ectemnostega quechua*, reported from Peru, Bolivia and northwestern Argentina, from Jujuy to Catamarca provinces (Morrone et al., 2004), is a characteristic inhabitant of small water bodies, either shallow or very shallow, with scarce or no vegetation, and variable temperatures (Bachmann, 1981).

The studied bivalves were nipped on adult males and females, either brachypterous or macropterous, as well as nymphs. In all cases, the sphaeriids were nipped on the tip of the middle legs of the corixids that bear two long and slender tarsal claws that are used by the insects to hold themselves to the bottom of the waterbody while resting (Fernando, 1954; Bachmann, 1981).

As it was reported by Darwin (1882), Standen (1885), Kew (1888, 1893), and Lansbury (1955) for the hemipterans *Nepa* and *Corixa punctata*, most of the *Ectemnostega* specimens here studied carried a single fingernail clam. On the other hand, Kew (1893) and Cash (1912) found some specimens of *Nepa* and *Corixa* carrying two bivalves, as herein reported. Kew (1893) even reported up to three bivalves clipped to a British corixid.

Darwin (1882), Green & Figuerola (2005) and Wood et al. (2008) reported that the attachment by bivalves usually damages the toes of amphibians and birds, or the legs of insects. This kind of damage was also observed in the case of *E. quechua*, whose middle leg tarsal claws appear broken at the tip. On the other hand, fingernail clams close their valves so tightly on the claws of corixids that their shell margins are injured (Fig. 5). This injury in the bivalve shell has not been previously reported.

The extensive list of aquatic insects reported as bearing sphaeriids nipped on their bodies reveals the potential of this relationship for bivalve dispersal. In this sense, it should be noted the great mobility usually exhibited by heteropterans. *Corixa punctata* and *Sigara lateralis*, for example, are known to have high migration rates (Brown, 1951; Fernando, 1954). Popham (1952) estimated that *Sigara striata*, *S. germari* and *S. nigrolineata* could migrate

about 10–13 km on a windless day, and *Corixa punctata* may have an even greater range. Unfortunately, nothing is known about the mobility of *E. quechua*. Some species, such as *Sphaerium corneum* (Linnaeus) may remain attached for up to six days on the legs of the coleopteran *Dytiscus marginalis* (Linnaeus) (Darwin, 1882). The great mobility and gregarious habits of corixids, and the long time of attachment reported for some sphaeriids to insects, pleads for them as suitable dispersal agents. The occurrence of closely located ponds could favour a stepping stone colonization. Since corixids abandon their habitats when they dry up, or when temperatures get too high, or for dispersal purposes, mainly during the night (Bachmann, 1981), such behaviours may also favour bivalve survival during transport. The finding of bivalves clipped to non-flying corixids (nymphs and brachypterous adults) suggests that this relationship does not necessarily imply the dispersal between different aquatic habitats (lateral dispersal), but their relocation to deeper waters within the same waterbody, when the habitat starts to dry. Corixids are fast swimmers that move regularly from the bottom to the surface to renew the atmospheric air reserve (plastron) that they carry below the hemelytra (Bachmann, 1981). Therefore, in situ movements of corixids could favour sphaeriids at finding more suitable microhabitats. It cannot be dismissed that shell closing and clamping of *S. lauricochae* is automatically triggered by any moving object (small or large, live or inert) that touches the bivalve. Although not always successful in securing long distance dispersal, such an evolutionary fixed response would still be adaptive for sphaeriid survival.

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A NEW SPECIES OF *MYSELLA* ANGAS, 1877
FROM SOUTHERN CHILE (BIVALVIA: GALEOMMATOIDEA)

Marina Güller^{1,2*} & Diego G. Zelaya^{2,3}

INTRODUCTION

The Patagonian fjord region, extending from Puerto Montt (41.5°S) to Cape Horn (56°S), is the most diverse but also the most neglected part of the Chilean coast (Häussermann & Försterra, 2009). With a total of 60 currently known species, bivalves are relatively well-represented, although Zelaya (2009) pointed out that the diversity of this group could be significantly increased when more detailed studies, particularly those considering small-sized species, are done. The checklist for the area includes currently only one species of *Mysella*, *M. mabillei* (Dall, 1908). This contribution deals with the description of a second, new species of *Mysella* from the area, and its comparison with the other species of the genus currently known from Chilean waters.

MATERIAL AND METHODS

The specimens here studied were obtained between 10 and 113 m deep, by using a 2 mm mesh-size net, sorted from the sediment under a stereoscopic microscope, and deposited at the Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN), Buenos Aires and the Museo de La Plata (MLP), La Plata.

Specimens were studied and illustrated with scanning electron microscopy (SEM). Shell length (L) refers to the maximum antero-posterior distance; shell height (H) to the maximum dorso-ventral distance, perpendicular to L; and shell width (W) to the maximum distance across united valves. The mean, standard deviation, and number of specimens measured (n) for the ratio H/L and W/H, are given. Anatomical information was obtained from Raillet-Henry decalcified specimens, dissected under stereoscopic microscope.

SYSTEMATICS

Mysella cahuelmensis, n. sp.
(Figs. 1–13)

Type Locality: 42°15'22"S, 72°23'48"W, Fiordo Cahuelmo, Chile, 10–30 m.

Type Material: Holotype (MACN-In 38938) and 14 paratypes from the type locality (5 specimens and 4 coated embryos, MACN-In 38939; 4 specimens and 1 valve, MLP 13545).

Other Material Examined: 2 specimens, 2 valves, 42°15'24"S, 72°24'10"W, Fiordo Cahuelmo, Chile, 50–95 m (MLP 13546); 1 specimen, 42°15'37"S, 72°25'52"W, Fiordo Cahuelmo, Chile, 70–113 m (MACN-In 38940).

Diagnosis: Shell ovate, flat; with coarse com-marginal cords; anterior end produced; dorsal margin short, straight to slightly curved; ventral margin projecting anteriorly; beaks inflated, posterior to midline.

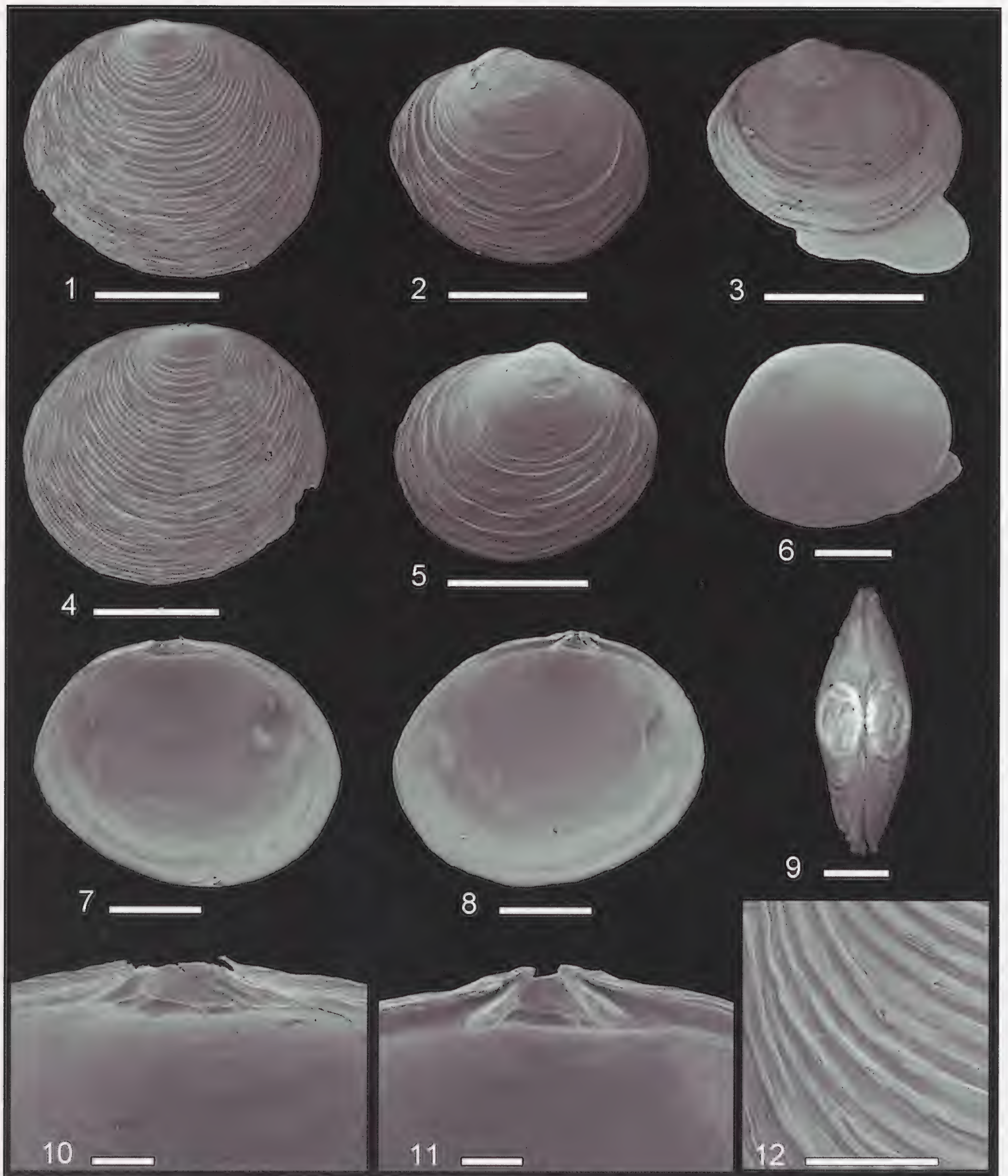
Description: Shell relatively large for genus (maximum observed length = 5 mm), ovate (H/L = 0.82 ± 0.05 mm, n = 13), compressed (W/H = 0.46 ± 0.06 mm, n = 13), thin, white (Figs. 1–5, 7–9). Anterior end produced, more markedly in small-sized specimens; posterior end shorter and lower than anterior (Figs. 1–5, 7, 8). Anterior and posterior parts of dorsal margin short, similar in length, straight, horizontal to slightly sloping (Figs. 1–5, 7, 8). Anterior margin widely curved, forming a weak angle at junction with dorsal margin, broadly rounded ventrally. Ventral margin projecting anteriorly. Ventral part of posterior margin continuous with ventral margin; dorsal part obliquely

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FIGS. 1–12. *Mysella cahuelmensis*, new species. FIGS. 1, 4, 12: Holotype (MACN-In 38938); FIGS. 2, 5–11: Paratypes (MACN-In 38939); FIG. 3: Specimen from the type locality, decalcified for anatomical studies; FIGS. 1–5: Outer views; FIGS. 1–3: Right valve; FIGS. 4, 5: Left valve; FIG. 6: Larva removed from an adult; FIGS. 7, 8: Inner views; FIG. 7: Left valve; FIG. 8: Right valve; FIG. 9: Dorsal view; FIGS. 10, 11: Detail of hinge plate; FIG. 10: Left valve; FIG. 11: Right valve; FIG. 12: Detail of shell sculpture. Scale bars: FIGS. 1, 4 = 2 mm; FIGS. 2, 3, 5, 7, 8 = 1 mm; FIGS. 6, 10, 11 = 200 μ m; FIGS. 9, 12 = 500 μ m.

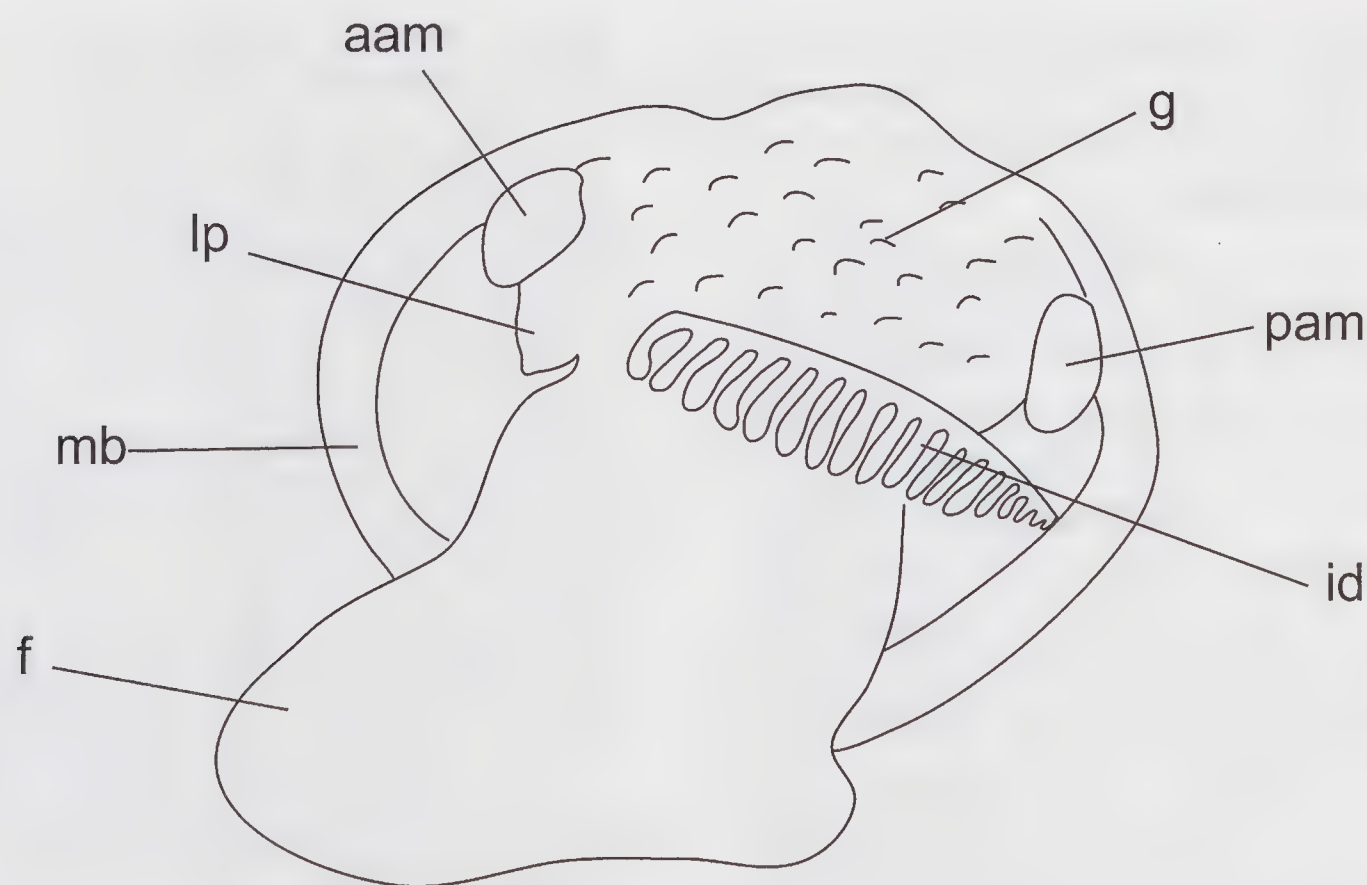


FIG. 13. *Mysella cahuelmensis*, new species, anatomy. aam: anterior adductor muscle; f: foot; g: gonad; id: inner demibranch; lp: labial palp; mb: mantle border; pam: posterior adductor muscle.

truncate, forming weak angles at junctions with ventral part and dorsal margin (Figs. 1–5, 7, 8). Beaks inflated, located posterior to mid-line, usually eroded in the studied specimens, opisthogyrous (Figs. 1–5). Prodissoconch ovate, smooth, about 570 μm in diameter (Fig. 6). Shell surface sculptured with coarse, regularly separated commarginal cords (Fig. 12). Periostracum brown to yellowish-brown, sometimes crusted with ferruginous material. Inner shell surface white, mimicking outer shell sculpture. Hinge plate narrow, but well visible beneath beaks (Figs. 7, 8, 10, 11). Right valve with two straight, divergent, equally solid teeth; the anterior slightly longer than the posterior. Anterior to anterior tooth and posterior to posterior tooth, hinge plate margin forming a low ridge, delimiting with dorsal shell margin two grooves to accommodate margin of opposite valve (Fig. 11). Left valve edentulous, with dorsal margin moderately thickened on each side of ligamental pit, forming short lamellae that interlock with the grooves of right valve. Ligamental pit deeply recessed, flanked by anterior and posterior ligamental ridges (Fig. 10). Ligament large, internal.

Anatomy: Mantle margin with long inhalant-pedal aperture and small posterior exhalant aperture. Transverse section of anterior and posterior adductor muscles ovate, similar in size. Gill axis obliquely directed (Fig. 13).

Only one (the inner) demibranch, present, with 19 filaments in a 2 mm length specimen (Fig. 13). Ascending and descending lamellae equally developed. Left and right demibranchs posteriorly fused. Foot large, compressed, with well-marked posterior heel (Fig. 13). Brooding was observed in a 4.3 mm long specimen.

Ethymology: The species name refers to the collection site, the Fiordo Cahuelmo, which in Mapuche aboriginal language means “place of cahueles”; “cahuel” is the name used to refer to the Peale’s Dolphin, *Lagenorhynchus australis* (Peale, 1848).

Remarks: The new species here described shows a combination of characters reported for *Mysella* – with *Rocheffortia* Vélain, 1877, considered a synonym, by Gofas & Salas (2008) – and *Kurtiella* Gofas & Salas, 2008. It shares with *Mysella* the presence of a continuous cardinal platform under the beaks and the teeth of the right valve entirely supported by the cardinal platform, and with *Kurtiella* subequal teeth in the right valve. Gill morphology in *Mysella cahuelmensis* also resembles that described for *Kurtiella*, particularly that of *K. tumidula* (Jeffreys, 1866) (Gofas & Salas, 2008: fig. 14A). Although this character is unknown in the type species of either *Mysella* and *Rocheffortia*, a wide variation of gill development has been reported for other species currently assigned

to *Mysella* (Boyko & Mikkelsen, 2002). Due to these uncertainties, we prefer to be conservative and tentatively place the new species here described under *Mysella*.

Although the generic placement of Chilean mysellids is unresolved, six species of *Mysella* s. l., are currently known from the area, three from the central and northern coasts – *Mysella coquimbensis* (Hanley, 1857), *Mysella deanneae* Ramorino, 1968, and *M. molinae* Ramorino, 1968) – and three from the Magellan Strait – *Mysella mabillei* (Dall, 1908), *Mysella rochebrunei* (Dall, 1908) and *Mysella sculpta* Soot-Ryen, 1957. ***Mysella cahuelmensis*** resembles *M. deanneae* and *M. coquimbensis* in having an ovate shell outline, differing in having the anterior and posterior parts of dorsal margin shorter, similar in length, and horizontal to only slightly sloping, and the ventral margin more widely arcuate.

The evenly ovate shell outline clearly distinguishes ***Mysella cahuelmensis*** from *Mysella mabillei*, *M. rochebrunei*, *M. sculpta*, and *M. molinae*. The two former species have a rectangular shell outline, with the ventral margin straight and parallel to the anterior part of dorsal margin; and the two latter species have a trigonal-ovate shell outline. In addition, in *M. molinae* the right valve has a posterior tooth that is longer than the anterior tooth. ***Mysella cahuelmensis*** differs from any other mysellid occurring in Chilean waters in its coarse com-marginal sculpture.

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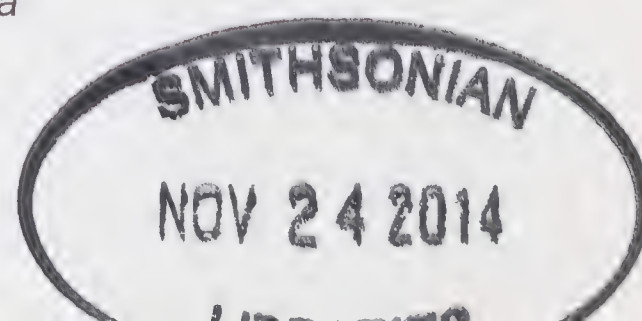
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Mr. Timm Haun
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Mr. Haun finished his diploma thesis at Goethe University, Frankfurt am Main, in 2010 on the topic published in his *Malacologia* paper. He is currently a PhD student of the Molecular Ecology group of Professor Marcus Pfenniger at the Biodiversity and Climate Research Center in Frankfurt. His present area of study is “The genomic basis of adaptation and niche evolution of *Candidula* species.”

Paula Mikkelsen

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Suzete R. Gomes^{1*}, David G. Robinson¹, Frederick J. Zimmerman²,
Oscar Obregón³ & Norman B. Barr³

ABSTRACT

In this study, we propose *C. confusus*, new species, an Andean slug of the genus *Colosius* Thomé, 1975, and a newly recognized pest of coffee and cultivated flowers from Colombia, Ecuador and Peru. We compare it with *C. pulcher* (Colosi, 1921), a poorly known species with which it has been confused. Our study is based on morphological analysis of a large number of specimens, including interceptions on cut-flowers and live plants by federal agricultural inspectors of the United States Department of Agriculture (USDA) and the Department of Homeland Security (DHS), and material from eight museum collections. Genetic diversity within *C. confusus*, n. sp. and *C. pulcher* is also analysed based on fragments of cytochrome oxidase I (COI), and 16S rRNA. They are differentiated by reproductive characters and genes studied. In *C. confusus*, n. sp., the phallus has a deep longitudinal groove from the base, near the retractor muscle, to its distal region, close to the papilla. In *C. pulcher*, there is an oval to rectangular swelling on the basal region of the phallus. Some important differences between these species are also found in the digitiform gland and bursa copulatrix. We describe, illustrate and discuss the color variation, morphological similarities, diagnostic characters and its variation, habitat and distribution for each species. Genetic diversity within *C. confusus*, n. sp., and *C. pulcher* is low. In order to analyze their relationship with *C. propinquus* (Colosi, 1921) (currently a junior synonym of *C. pulcher*) and *C. lugubris* (Colosi, 1921) (type-species of *Colosius*), fragments of COI, 16S, and 28S rRNA genes are also analyzed in a sample of these species. *Colosius confusus*, n. sp., is a distinct lineage within the genus *Colosius*. It is not a sister species of *C. pulcher*, which has *C. propinquus* as a sister species, here recognized as valid. *Colosius confusus*, n. sp., is closer to the clade that includes *C. pulcher* and *C. propinquus* than it is to *C. lugubris*. Based on the phylogenetic reconstruction, *C. lugubris* is sister to all the other *Colosius*, although additional studies are required to formally test phylogenetic placements and monophyly of the genus. Associated imports and number of interceptions per year of *C. confusus*, n. sp., by agricultural inspectors are also presented.

Key words: land slug, morphology, Ecuador, Peru, Colombia, 16S, COI, 28S.

INTRODUCTION

The slugs of the Veronicellidae Gray, 1840, have been classically distinguished from other pulmonates by the absence of a shell and a developed pulmonary cavity. They are found throughout the tropical and subtropical regions (Thomé, 1993). Some species are agriculturally significant causing serious damage and losses to numerous crops (Andrews & Pilz, 1985; Ca-

ballero et al., 1991; Thomé, 1993; Chiaradia & Milanez, 1999; Chiaradia et al., 2004; Robinson & Hollingsworth, 2005). Some are intermediate hosts of *Angiostrongylus costaricensis* Moreira & Céspedes, 1971, and *Angiostrongylus cantonensis* (Chen, 1935), nematodes responsible for abdominal angiostrongyliasis and eosinophilic meningoencephalitis, respectively, which can accidentally infect humans (Graeff Teixeira et al., 1989, 1994; Rambo, 1997; Laitano et

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al., 2001; Caldeira et al., 2007; Ohlweiler et al., 2010). Despite their agricultural and public health importance, studies of their systematics, distribution and biology are still lacking for most species of the family.

Colosius Thomé, 1975 is one of the eighteen currently recognized genera of the Veronicellidae from the Americas. According to Thomé (1975), it is reported in Ecuador, Peru, Colombia, the Dominican Republic and Haiti and consists of five species: *C. buergeri* (Simroth, 1914) (described from the Dominican Republic), *C. festae* (Colosi, 1921) (described from El Pun, Ecuador), *C. lugubris* (Colosi, 1921) (type-species from Quito, Ecuador), *C. propinquus* (Colosi, 1921) (described from El Pun, Ecuador) and *C. pulcher* (Colosi, 1921) (described from Quito, Ecuador). In the diagnosis provided by Thomé (1975) for the genus, he described as features shared by these species: the rectum penetrating in the tegument close to the female genital pore; the presence of a digitiform gland, with tubules differentiated in two sets, one with short and unbranched tubules and another with long and branched tubules; an oval or globular bursa copulatrix without a “cabecote” (= like a small tip in the bursa proper where the vas deferens penetrates), a short and, in general, thick duct; a short canalis junctor that penetrates in the bursa proper, next to the duct or in the duct, auxiliary to the bursa; and lack of any accessory structure.

In this study our main objective was to describe morphologically a new species of *Colosius*, currently the second most intercepted mollusk species in Miami by federal agricultural inspectors in cut-flowers from Ecuador and Colombia. A secondary objective was to compare it with *C. pulcher*, a species known only from its original description (Colosi, 1921) and re-description of its lectotype (Thomé, 1970b), with which it has been confused. We describe, illustrate and discuss the color variation, morphological similarities, diagnostic characteristics and variation, habitat and distribution for both species. Levels of genetic divergence between species and genetic diversity for them are also analyzed based on the fragments of cytochrome oxidase I (COI) and 16S rRNA genes. Their relationship with *C. propinquus* (Colosi, 1921) (currently regarded as a junior synonym of *C. pulcher*) and *C. lugubris* (Colosi, 1921) (type-species of *Colosius*) are also investigated based on COI, 16S and 28S rRNA. Imports in which specimens were intercepted and number of federal interceptions per year for *C. confusus* are presented.

MATERIALS AND METHODS

Morphological Analysis

A total of 281 specimens intercepted by federal agricultural inspectors were morphologically analyzed. They are deposited in the USDA's National Malacology Laboratory collection at the Academy of Natural Sciences of Drexel University in Philadelphia (Pennsylvania, U.S.A.), and in the USDA Miami Plant Inspection Station collection (Florida, USDA). Eleven lots of *C. confusus* collected in Peru and Ecuador and four lots of *C. pulcher* collected from Ecuador were also analyzed and are deposited in the same collection. Lots from the following institutions were also analyzed: Museo Regionale di Scienze Naturali di Torino (MSNT) (Torino, Italy) (paralectotypes), National Museum of Natural History, Smithsonian Institution (USNM) (Washington, D.C., U.S.A.), Florida Museum of Natural History (FMNH) (Gainesville, Florida, U.S.A.), Zoological Museum University of Copenhagen (ZMUC) (Copenhagen, Denmark, Museo de Zoología, Pontificia Universidad Católica del Ecuador (QCAZ-M) (Quito, Ecuador), Natural History Museum of the National University of San Marcos (MUSM) (Lima, Peru) and Superintendência de Controle de Endemias do Estado de São Paulo (CMS-DPE) (São Paulo, Brazil). Among these specimens, 70 correspond to *C. confusus* (besides 281 specimens from interceptions in August 20th, 2012) and 154 specimens correspond to *C. pulcher*. Two specimens of *C. propinquus* (QCAZ-M-0 03700 and QCAZ-M-003659), and one of *C. lugubris* (USDA-110426) were also analyzed.

In order to analyze the internal morphology, each specimen was dissected under a Nikon® SMZ1500 stereomicroscope. The numbers of tubules of the digitiform gland was determined from 40 randomly selected specimens of *C. confusus*, n. sp., and *C. pulcher*. Digital images of the diagnostic structures were obtained using a Nikon® digital Sight DS-Fi1 camera attached to the Nikon® SMZ1500 stereomicroscope. Multiple images were combined using Helicon Focus 4.03 Pro to increase depth of field, and subsequently histogram normalized using Adobe® Photoshop CS5. Drawings based on these photographs were made using CorelDraw® X5. Morphological characteristics described and illustrated for the species were those considered diagnostic for Veronicellidae (Hoffmann, 1925; Coifmann, 1935; Forcart, 1953; Thomé, 1970a, b, 1972, 1975; Gomes & Thomé, 2001).

Distribution

Records of occurrence, geographical coordinates and altitude were obtained from the lots examined and literature (Colosi, 1921; Hoffmann, 1925; Thomé et al., 1997; Thomé et al., 2001). Coordinates and altitude not obtained by the collectors were found using GoogleMaps. Coordinates were plotted on maps using the program Versamap version 2.07.

Federal Interceptions

Data related to interceptions of *C. confusus*, n. sp., including country of origin, date of interception and type of import in which the specimen was intercepted were obtained from the Agriculture Quarantine Activity Systems (AQAS) of the Plant Protection and Quarantine (PPQ) of the Animal and Plant Health Inspection Service (APHIS), of the United States Department of Agriculture (USDA), based on 281 records of interception obtained as of August 20th, 2012.

Molecular Analysis

From the intercepted and field collected material and recently collected material, a subset was selected for DNA analysis. This included 59 specimens of the new species *C. confusus* from Colombia, Ecuador and Peru and eight specimens of *C. pulcher*, four specimens of *C. lugubris* and two specimens of *Colosius propinquus* from Ecuador, besides six specimens representing outgroups to *Colosius* (Table 1).

DNA was isolated from samples using the DNeasy tissue extraction kit of Qiagen (Valencia, California, U.S.A.). Foot tissue was cut from each specimen using a sterile razor blade and then incubated at 56°C in ATL buffer containing Proteinase K (Qiagen) following the manufacturer's instructions. The foot tissue typically dissolved after one hour of incubation, but incubation times ranged from 1 to 14 hours. DNA was eluted from Qiagen columns using 50–100 µl buffer (Qiagen) and was stored at 4°C until analysis and then at -20°C after analysis. Voucher specimens are deposited in the collection of the USDA's National Malacology Laboratory collection at the Academy of Natural Sciences of Drexel University, in Philadelphia, Pennsylvania, U.S.A., and the Museo de Zoología, Pontificia Universidad Católica del Ecuador, in Quito, Ecuador.

The COI, 16S, and 28S gene fragments were amplified using four separate PCR reac-

tions. The 5' region of the cytochrome oxidase c subunit I (COI) gene commonly used for DNA barcoding of animals was amplified using the primers LCO-1490 (5-GGTCAA-CAAATCATAAAGATATTGG) and HCO-2198 (5-TAAACTTCAGGGTGACCAAAAAATCA) (Folmer et al., 1994). The 16 rRNA gene fragment was amplified using 16S-1 (5- CGC-CTGTTTAACAAAAACAT) and 16S-2 (5-ACGC-CGGTTTGAAGCTCAGATC) (Barr et al., 2009). Two separate, non-overlapping fragments were amplified from the 28S rRNA gene. The first 28S fragment included the expansion segment D2. This region was amplified from *C. confusus*, n. sp., (Accession, BX090401-019) using Wade & Mordan's (2000) LSU-2 (5-GGGTTGTTTG-GGAATGCAGC) and LSU-4 (5-GTTAGACTC-CTTGGTCCGTG) primers located in conserved 28S regions C2 and C3, respectively. This primer pair generated multiple bands for several other mollusk samples so alternate primers were designed using an alignment of *C. confusus*, n. sp., *Pedipes mirabilis* (AY465074), and *Vaginulus (Sarasinula) plebeius* (DQ256745) (= *Sarasinula plebeia*). The new primer pair 28S-2F (5-CCGTGAGGGAAAGTTGAAAA) and 28S-4R (5-GTTGCCCAGTCTCTC-CCAGT) is located in C2 and D2 segments, respectively, and amplifies the majority of D2 segment. The fourth fragment amplified the 28S expansion segment D3 using the primers 28S-a (5- GACCCGTCTTGAAACACGGA) and 28S-b (5-TCGGAAGGAACCAGCTAC) located in conserved regions C3 and C4, respectively (Whiting et al., 1997). All four loci (three genes) were used to estimate phylogenetic relationships among species. Additional sequences of 16S and COI were generated for *C. pulcher* (JX629298–JX629311) and *C. confusus*, n. sp., (JX629312–JX629371). 16S locus was used to estimate genetic diversity among *C. confusus*, n. sp., and *C. pulcher* samples.

Reactions were either performed in 50 µl reactions using Ex Taq polymerase (Hot Start version, Takara Bio, Madison, Wisconsin, U.S.A.) or OmniMix HS lyophilized universal PCR reagent beads (Cepheid smart cycler systems, Takara Bio). The OmniMix protocol was used for samples that failed to amplify using the Ex Taq method. Reactions using Ex Taq had final reagent concentrations of 1X buffer, 0.2 mM each dNTP, 0.2 µM each primer, and 0.025 U/µl Taq enzyme. The OmniMix reactions had a final concentration of 0.2 µM of each primer. The Ex Taq and OmniMix protocols used 1 and 2 µl of DNA template, respectively. Water was used as a negative control in all PCR experiments. All

TABLE 1. List of the specimens and outgroups used in the phylogenetic analysis with sampling localities and year of collection, voucher-specimens and GenBank accession numbers.

Taxon	Country	Primary division	Year	Voucher	28S-D2	28S-D3	16S	COI
<i>Sarasinula plebeia</i>	Brazil	Rio Grande do Sul	2001	CMS-DPE 1053	JX532130	JX532142	JX532118	JX532107
<i>Sarasinula linguaeformis</i>	Brazil	Minas Gerais	2006	USDA 120073	JX532131	JX532143	JX532119	JX532108
<i>Semperula wallacei</i>	American Samoa	Tutuila	2005	USDA 110486	JX532132	JX532144	JX532120	JX532109
<i>Laevicaulis natalensis</i>	South Africa	Natal	1995	USDA 10095	JX532133	JX532145	JX532121	JX532110
<i>Phyllocaulis boraceiensis</i>	Brazil	São Paulo	1999	USDA 110485	JX532134	JX532146	JX532122	JX532111
<i>Veronicella cubensis</i>	Antigua	St. Paul Parish	2003	USDA 110484	JX532135	JX532147	JX532123	JX532112
<i>Colosius lugubris</i>	Ecuador	Pichincha	1997	USDA 110426	JX532136	JX532148	JX532124	None
Colosius confusus	Colombia	Intercept	2008	USDA 110483	JX532137	JX532149	JX532125	JX532113
Colosius confusus	Ecuador	Napo	2011	USDA 110422 B	JX532138	JX532150	JX532126	JX532114
<i>Colosius propinquus</i>	Ecuador	Imbabura	1999	QCAZ-M-003659	JX532139	JX532151	JX532127	JX532115
<i>Colosius pulcher</i>	Ecuador	Pichincha	2011	USDA 110419 A	JX532140	JX532152	JX532128	JX532116
<i>Colosius pulcher</i>	Ecuador	Napo	2011	USDA 110421 A	JX532141	JX532153	JX532129	JX532117

reactions were performed on Gene Amp 9700 PCR instruments (Applied Biosystems, Foster City, California, U.S.A.) using the following cycling parameters: 94°C (3 min), 35 cycles of 94°C (20 sec) / 50°C (20 sec) / 72°C (30 sec), and an extension step of 72°C (5 min).

PCR products were visualized on 1% TAE agarose gels stained with ethidium bromide and purified using either the QiaQuick purification kit (Qiagen) or ExoSAP-IT (USB Products, Affymetrix, Cleveland, Ohio) protocol according to the manufacturer’s instructions. All samples were sequenced on an ABI 3730xl DNA Analyzer (GeneWiz Inc., South Plainfield, New Jersey) in both directions using the forward and reverse primers.

The bidirectional trace files generated from the 3730xl were imported into Sequencer v5.0 (Gene Codes, Ann Arbor, Michigan) and edited by hand to generate a consensus file for each sample. The consensus files were imported into MEGA 5 (Tamura et al., 2011) and aligned first using MUSCLE and then adjusted by hand. Alignments are deposited in GenBank and available from the authors. The location of primers and regions of the 28S sequences were identified by comparison to secondary structure of *Apis mellifera* (Gillespie et al., 2006). Regions of ambiguous alignment in the ribosomal gene fragments were recorded and excluded from all subsequent phylogenetic analysis. All sites were used to estimate diversity within a species. The four loci were concatenated into a single alignment for phylogenetic analysis and model testing. Sequences were submitted to GenBank (JX532107–JX532153).

MEGA 5 was used to identify unique genotypes per locus and to estimate pairwise uncorrected genetic distances between *Colosius* specimens and species. Goodness-of-fit to models of evolution for each alignment was tested using corrected Akaike information criterion (AICc, Hurvich & Tsai, 1989) using jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008) and MEGA5 (Tamura et al., 2011). The jModelTest results selected the transversional model of Posada (2003; TVN + G + I) as the best-fit model and MEGA5 selected the General-Time-Reversible model (GTR + G) as the best-fit model. Based on the similarity of models and compatibility with phylogenetic programs, the GTR + G + I model was selected for subsequent analyses. Maximum Likelihood (ML) trees were generated using PAUP* (Swofford, 2002) and the GTR+G+I model [Lset base (0.2655, 0.1844, 0.2426), rmat (0.5739, 8.7561, 6.8296, 0.8290, 8.7561), shape (0.8570), ncat (4), pinvar (0.5610)].

The ML tree was generated for the concatenated data set using a branch and bound search with no limit on trees saved. An additional heuristic search using 100 bootstrap replicates each of 10 random additions and TBR swapping was performed to estimate branch support. Bayesian Inference (BI) was performed on the concatenated data set using MrBayes 3.1 and the model selected for the ML searches. Tree searches were performed using 1,000,000 generations and sampling every 500 generations. The first 1,000 trees were discarded as a burn-in and four chains were run with a heating parameter of 0.2. Convergence was estimated using the program diagnostic output and the average standard deviations of split frequencies was expected to be less than 0.01. The BI tree was reported as a majority-rule consensus using a 50% cut-off for Posterior Probabilities (PP) for branch support. Additional tree searches were performed on data partitions (16S, 28S-D2, 28S-D3, and COI) to compare with the concatenated tree search results. These were performed using maximum likelihood in PAUP (Heuristic search, 100 random additions and TBR branch swapping) using empirical frequencies and HKY model selected by jModelTest.

RESULTS

Molecular Data

DNA sequences of the four loci were generated for eleven of the twelve specimens included in the phylogenetic analysis (Table 1). The COI fragment consistently failed to amplify for the *C. lugubris* sample using the standard PCR conditions and modified PCR

TABLE 2. Minimum genetic pairwise differences between *C. confusus* and *Colosius* species.

	28S-D2	28S-D3	16S	COI
<i>C. confusus</i>	p-distance			
<i>C. pulcher</i>	0.014	0.014	0.105	0.118
<i>C. propinquus</i>	0.014	0.024	0.111	0.116
<i>C. lugubris</i>	0.010	0.015	0.150	NA
	No. Gap sites (No. INDELS)			
<i>C. pulcher</i>	0 (0)	7 (2)	11 (5)	3 (1)
<i>C. propinquus</i>	0 (0)	4 (2)	12 (6)	3 (1)
<i>C. lugubris</i>	0 (0)	19 (4)	9 (6)	NA

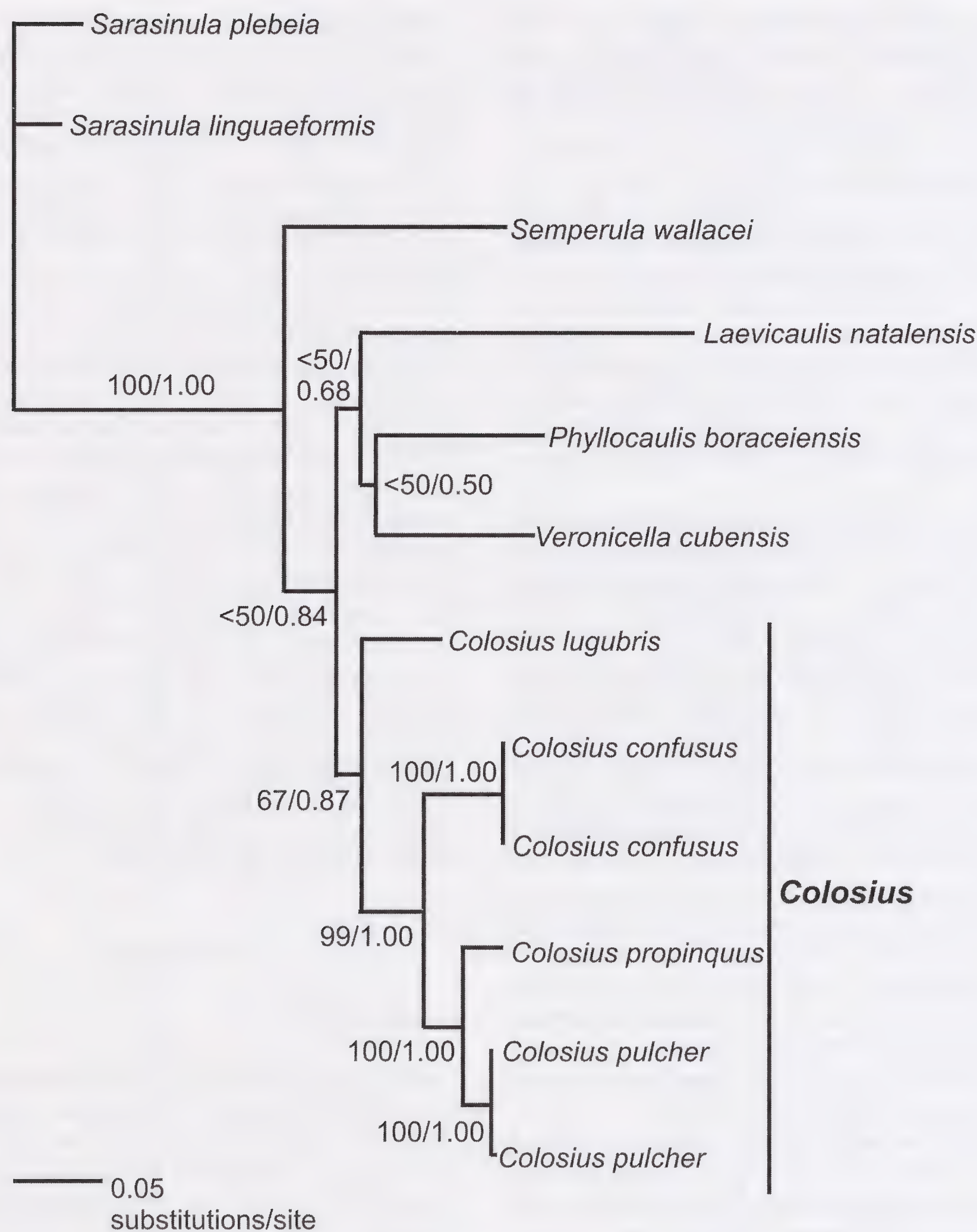


FIG. 1. Best tree identified with branch and bound search for the Maximum Likelihood (score 6023.68061) obtained from the combined cytochrome oxidase I (COI), 16S rRNA, and 28S rRNA) (1593 bp). Numbers above and below nodes are branch support values measured as ML bootstrap and BI posterior probabilities, respectively.

conditions (e.g., increased cycles and lower annealing temperatures). DNA isolated from other *C. lugubris* specimens also failed to amplify the COI and 28S loci. These three *C. lugubris* isolates did generate 16S sequences (JX629303–JX629305) but were not included in further analysis.

As expected for ribosomal genes, segments of the 28S and 16S genes were highly divergent among species and it was difficult to assign

homology to these regions of the alignment. Consequently, those regions were excluded from phylogenetic analysis and model selection but used to calculate pairwise differences among *Colosius* specimens. A final alignment of 1593 bp was generated after concatenation of 28S-D2 (295 bp), 28S-D3 (308 bp), 16S (408 bp), and COI (582 bp). A total of 109 sites were excluded from analyses. These sites are reported here for the concatenated data set and

for each locus in parentheses: 380–423 (28S-D3: 85–129), 618–624 (16S: 15–21), 808–832 (16S: 205–229), 863–867 (16S: 260–264), and 878–905 (16S: 275–302). The percentage of invariant sites in the concatenated data set was highest for the 28S loci (c. 90%) and between 50–60% for the mitochondrial loci.

Uncorrected genetic difference (p-distance) between *Colosius* specimens ranged from 0 to 15.7% for 16S. The minimum distance between different species was 4.1% (between *C. pulcher* and *C. propinquus*). *Colosius lugubris* was the most divergent of the species having minimum p-distances of 14–16% in comparison to the other *Colosius* species. Divergence values for the COI gene ranged from 6.5–11.6% but no data were available from *C. lugubris* for comparison. ***Colosius confusus***, n. sp., had a deletion of an amino acid (3 nucleotides) not shared in the other species. Minimum genetic diversity values between ***C. confusus***, n. sp., and the other *Colosius* species for each gene partition is given in Table 2. This table includes the number of observed gap sites (bases) and insert/deletion (INDEL) events between species.

Only one 16S haplotype was observed in an analysis of 59 ***C. confusus***, n. sp., samples. Analysis of five ***C. confusus*** samples revealed two COI haplotypes that were divergent by 0.2%. Four 16S haplotypes ($\leq 1.2\%$ p-distances) were observed in six samples and two COI haplotypes (0.3% p-distance) were observed in eight samples of *C. pulcher*. Two ***C. confusus***, n. sp., and two *C. pulcher* samples were selected for the phylogenetic analysis (Table 1) in order to consider the diversity found within these species.

A branch and bound search for the Maximum Likelihood tree identified one best tree (score 6023.68061; Fig. 1). This topology was also recovered in the Majority-rule Bayesian Inference tree. Branch support values measured as ML bootstrap and BI posterior probabilities are reported in Figure 1. Each one of the six species of *Colosius* form a monophyletic lineage in the tree. The 28S, 16S, and COI genotypes sequenced from ***C. confusus***, n. sp., samples are distinct from genotypes sequenced from *C. pulcher* and other species of *Colosius* studied (*C. lugubris* and *C. propinquus*). Branch support values are not high for this relationship ($< 80\%$ bootstrap and < 0.90 PP) but it is the best estimate given the available data. According to our phylogenetic results, ***C. confusus***, n. sp., is not the sister-species of *C. pulcher*

(Fig. 1). ***Colosius confusus***, n. sp., is sister to the clade that includes *C. pulcher* and *C. propinquus*. The type species in the genus, *C. lugubris*, is sister to the other *Colosius* species in the study.

ML searches performed on partitioned data resulted in ML trees with different topologies (data not shown). The ML 16S partition tree (score 1837.48372) recovered a *Colosius* topology similar to the combined data set (Fig. 1), but *Semperula wallacei* was included as sister to ***C. confusus***, n. sp. The ML 28S-D2 partition tree (score 610.98971) recovered a monophyletic *Colosius* but with reduced resolution at branches connecting ***C. confusus*** n. sp. to its sister taxon (*C. propinquus* + *C. pulcher*). The 28S-D3 partition tree (score 457.50973) did not recover a monophyletic *Colosius* because *C. lugubris* was not recovered as the sister taxon to the other *Colosius* species. The ML COI partition (score 3212.11170) recovered the topology in Figure 1 but did not include data from *C. lugubris*.

SYSTEMATIC DESCRIPTION

Colosius Thomé, 1975

Colosius confusus, new species
(Figs. 2–7, 12)

Colosius pulcher. Constantino et al., 2010: 1–8 (from Neira, Colombia), *non* Colosi, 1921.

Holotype

ANSP-A22109: Ecuador, Tungurahua, Baños de Agua Santa, W 78°25'24", S 01°23'40", 2,000 m, 11.XII.2004, Col. A. Santillan.

Paratypes

ANSP-A22110: Ecuador, Napo, Cantón Quijos, Bairro Baeza Colonial, N 00°27'32.70", W 077°53'09.78", 1,860 m, 20.IX.2011, Col. S. R. Gomes et al., 1 ex.; CMS-DPE-107'6: Colombia, C'aldas, Neira, 1,700 m, 01.VII.2009, Col. Constantino et al., 7 ex.; QCAZ-M-003744: Ecuador, Chimborazo, Alausí, 26.XII.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003705: Ecuador, Cotopaxi, Otonga, N 00°19'11", W 78°57'00", 2,000 m, 30.VII.2000, Col. D. Alvarado, 1 ex.; QCAZ-M-003813: Ecuador, Napo, Baeza, XI.1985, 1,800 m, Col. Briones, 1 ex.; FMNH- 449321:



Peru, Piura, 11 mi. E Canchaque, on road to Huancabomba, 11.IV.1970, Col. K. Campbell, 1 ex.; MRSN-M492: Ecuador, Pichincha, Volcan Pululahua, N 00°02'16.14", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 1 ex.

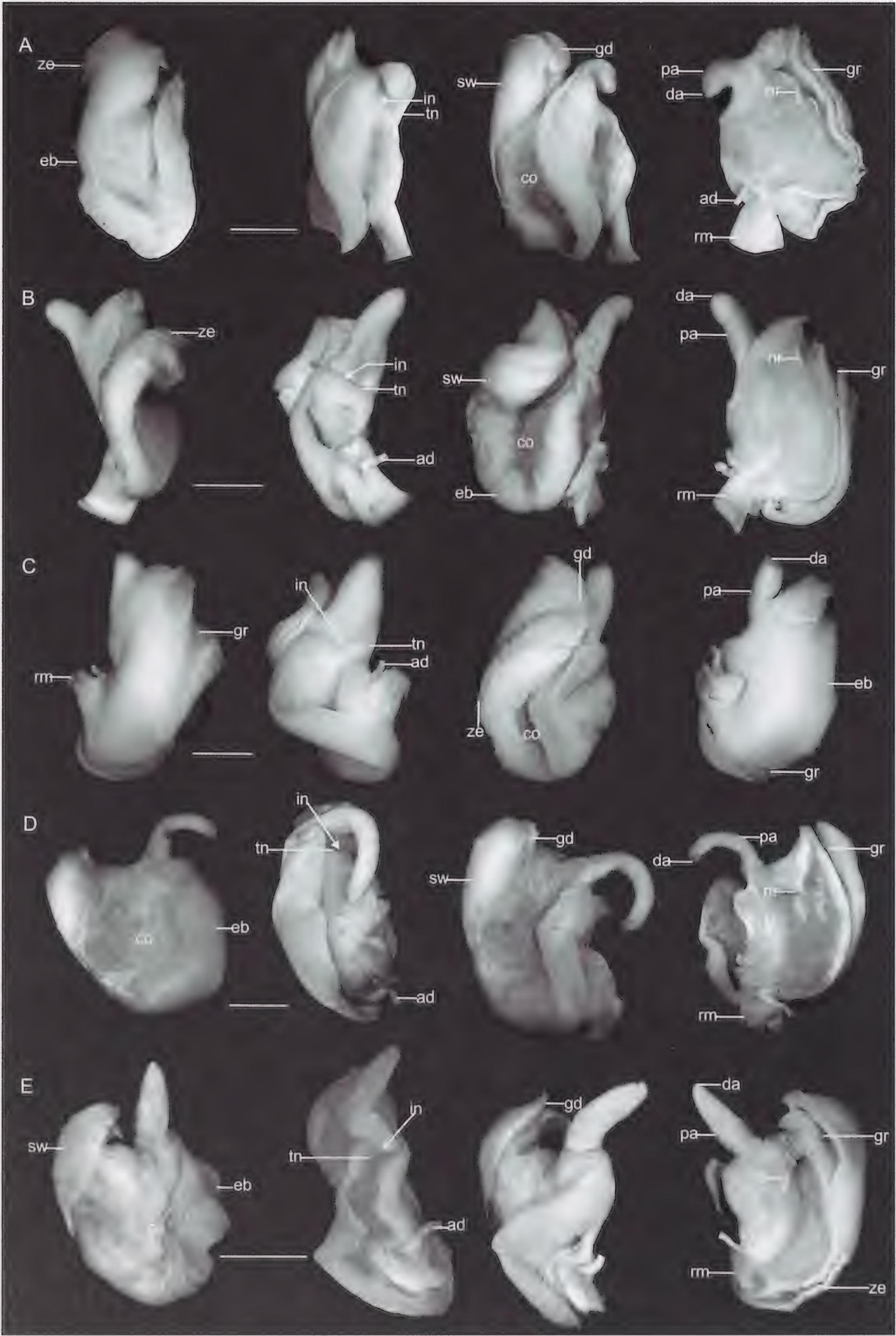
Other Material

QCAZ-M-003657: Ecuador, Imbabura, Caranqui, W 78°07', N 00°19', 3,000 m, 11.XII.1999, Col. C. Lara, 1 ex.; QCAZ-M-003660: Ecuador, Imbabura, Caranqui, N 00°19', W 78°07', 3,000 m, 11.XII.1999, Col. C. Lara, 1 ex.; QCAZ-M-003711: Ecuador, Cotopaxi, Otonga, N 00°25', W 79°00', 2,000 m, 09.I.2003, Col. M. C. Vizcaino, 1 ex.; QCAZ-M-003723: Ecuador, Cotopaxi, Otonga, 2,000 m, 09.I.2003, Col. M. F. Yauri, 1 ex.; QCAZ-M-003731: Ecuador, Cotopaxi, Otonga, N 00°28', W 79°00', 2,000 m, 09.I.2003, Col. M. C. Vizcaino, 1 ex.; QCAZ-M-003745: Ecuador, Chimborazo, Alausí, 02.IX.2000, Col. M. Reinoso, 1 ex.; QCAZ-M-003748: Ecuador, Chimborazo, Alausí, 26.XI.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003750: Ecuador, Chimborazo, Alausí, 23.XI.2000, Col. C. Landeta, 1 ex.; QCAZ-M-003755: Ecuador, Tungurahua, Patate, 23.XI.2000, Col. M. C. Erazo, 1 ex.; QCAZ-M-003758: Ecuador, Bolívar, San Miguel, 20.XII.1991, Col. A. Barragán, 1 ex.; QCAZ-M-003811: Ecuador, Napo, Cuyuja, 18.I.2003, N 00°29'12", W 70°00'48", 2,400 m, Col. R. D. Jadrin, 2 ex.; QCAZ-M-003815: Ecuador, Napo, Cosanga, 07.XII.1986, Col. M. Gavilanes, 2 ex.; QCAZ-M-003820: Ecuador, Napo, Cosanga, XII.1986, Col. J. Naranjo, 1 ex.; QCAZ-M-003821: Ecuador, Napo, El Chaco, 26.I.1993, Col. E. Bejarano, 1 ex.; QCAZ-M-003826: Ecuador, Napo, Cuyuja, 18.I.2003, 2,400 m, N 00°29', W 70°00', Col. F. Gonzales, 1 ex.; QCAZ-M-003828: Ecuador, Napo, Cuyuja, 18.I.2003, 2,400 m, N 00°29', W 70°00', Col. F. Gonzales, 1 ex.; USDA-110422: Hosteria Llacta Termale, Papallacta, Napo,

Ecuador, S 00°21'46.08", W 078°08'5.68", 3,300 m, 20.IX.2011, Col. S. R. Gomes et al., 2 ex.; USDA-110424: Ecuador, Pichincha, Volcán Pululahua, N 00°02'16.14", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 2 ex.; USDA-110425: Ecuador, Imbabura, Ibarra, BP Guayabilla, 2,451 m, 01.V.2011, Col. M. Correoso, 2 ex.; USDA 110705: Peru, Piura, Canchaque, S 05°22'19.6", W 079°36'03.2", 1,302 m, 02.VI.2012 Col. S. R. Gomes & V. Jiménez, 9 ex.; USDA 110706: Peru, Piura, Canchaque, S 05°22'16.5", W 079°35'53.9", 1,365 m, 02.VI.2012, Col. S. R. Gomes & V. Jiménez, 1 ex.; USDA-110707: Peru, Piura, Canchaque, S 05°21'22.3", W 079°35'26.8", 1,546 m, 04.VI.2012, Col. S. R. Gomes & V. Jiménez, 6 ex.; USDA-110713: Peru, Amazonas, La Florida, S 05°49'31.0", W 077°58'18.6", 2,220 m, 10.VI.2012, Col. C. Bebbini, 3 ex.; USDA-110715: Peru, Amazonas, La Florida, S 05°49'30.9", W 077°58'2.7", 2,220 m, 09.VI.2012, Col. S. R. Gomes & R. Ramirez, 5 ex.; USDA-110716: Peru, Amazonas, Leymebamba, S 06°42'32.6", W 077°48'17.9", 2,264 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2 ex.; MUSM-5557: Peru, Amazonas, Leymebamba, S 06°42'18.2", W 077°48'16.4", 10.VI.2012, 2,200 m, Col. S. R. Gomes & R. Ramirez, 1 ex.; USDA-110721: Peru, Amazonas, Leymebamba, S 06°42'57.4", W 077°47'57.3", 2,200 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2286, 1 ex.; USDA-110722: Peru, Amazonas, Leymebamba, S 06°43'00.2", W 077°48'10.9", 2,316 m, 10.VI.2012, Col. S. R. Gomes & R. Ramirez, 2 ex.; MUSM-5566: Peru, Hacienda Monterezo, Cajamarca, 2,200 m, 17–19.X.2009, Col. L. Figueroa & J. Gragos, 2 ex. Due to the large number of specimens of ***C. confusus***, n. sp., from USDA interceptions (281 to date), mostly from Colombia, the codes related to these specimens are not listed here. They are included at the USDA's Agricultural Quarantine Activity Systems database.

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FIG. 2. External color variation in ***Colosius confusus***, n. sp. A: Dorsal view of an adult specimen from Volcán Pululahua, Pichincha, Ecuador (USDA-110424A); B: Dorsal view of a young specimen intercepted on September 8th, 2010 (USDA-110446); C: Dorsal view of an adult specimen intercepted in Miami from Colombia on March 14th, 2011 (USDA-110448); D: Ventral view of the specimen shown in A; E: Dorsal view of specimen from Cantón Quijos, Napo, Ecuador; F: Dorsal and ventral view of specimens from Canchaque, Piura, Peru, found together at the base of leaves of plantain; G: Dorsal view of specimen from Neira, Manizales, Colombia (Constantino et al., 2010); H: Dorsal view of specimen from Leymebamba, Amazonas, Peru (USDA-110716A); I: Ventral view of the specimens shown in H. ca - bands without or with less spots often present on each side of the lighter line of the notum, that together form sometimes a lozenge-shaped area; bl - lines of black spots that may be present delimiting the lozenge-shaped area; fp - female genital pore; fo - foot; ki - keel; yl - lighter line located longitudinally and centrally on the notum; ys - yellow spots commonly found on the notum.



Etymology

The specific epithet refers to the confusion caused by the misidentification of the species of Veronicellidae with the phallus formed by an expanded muscular base, with two lateral brims, and by a conical and distal papilla. They include ***C. confusus***, *C. pulcher* and new species from Peru, which will be described in a paper currently in preparation. In these species, base and papilla are also delimited from each other by a transversal ridge in the ventral side of the phallus, that also it borders a shallow invagination (Figs. 3, 9, 14).

Diagnosis (Fig. 3)

The species has a phallus formed by an expanded base, with two lateral brims, and by a conical and distal papilla. Both (base and papilla) are delimited from each other by a transversal ridge that also delimits a shallow invagination in the ventral part of the phallus. One of these brims is much more developed than the other and has a deep groove from the phallus base (close to the retractor muscle) to its papilla. On the dorsal part of the phallus, mainly from its $\frac{1}{2}$ distal, a whitish swelling is commonly found along the margin of the groove. On each side of the groove along its entire length, a zigzag raised thread is visible. Numerous fine raised threads are scattered throughout nearly the entire dorsal side of the phallus. A few ridges are, in general, visible near the right distal portion of the groove, close to the papilla on the dorsal side.

External Morphology (Fig. 2)

Notum and hyponota background are brown, ranging from light to dark brown, or reddish-brown. Randomly distinct yellow or beige speckles may occur on this background (Fig. 2A–C, E). In the reddish-brown form, they are almost completely absent or not evident (Fig. 2H). Few black spots may be present, although in general they are sparse. A thin paler line is often centrally and longitudinally present (Fig. 2A, B, H), although its occurrence is variable. It may be absent, non-continuous or visible only near the posterior region of the body, especially in dark brown specimens. A well-defined keel is often present on this line, especially in contracted live specimens (Fig. 2E, G). Two brown bands without or with lighter spots are often present on each side of this paler line, which together can form a lozenge-shaped area (Fig. 2A, B, E, F). This area may be delineated by two lines of black spots (Fig. 2B, H), and barely visible or absent in dark brown specimens or in the reddish-brown form, where only the lines of black spots are present, but not the brown bands (Fig. 2G, H). The hyponota generally have color and intensity similar to the notum, but without the lighter spots observed on the notum (Fig. 2D, F, I). A pale narrow band may be longitudinally present along each side of the foot. The foot is yellow (with different shades of yellow) and is almost the same width as each hyponotum (Fig. 2D, F, I). The female genital pore is located very close to the pedal groove (around 1.5 mm from it) (Fig. 2D). Ocular tentacles are bluish-gray and brown at the tip.

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FIG. 3. Phallus variations of ***Colosius confusus***, n. sp. Five different specimens (A–E) with the phallus in four distinct positions. The first position is the dorsal view, the second is the ventral view and the others are lateral views of the phallus. A: Specimen intercepted from Colombia on August 5th, 2010 (USDA-110447); B: Specimen intercepted from Colombia on June 10th, 2010 (USDA-110449); C: Specimen intercepted from Colombia on March 13th, 2011 (USDA-110448); D: Specimen intercepted from Colombia on November 8th, 2008 (USDA-110450); E: Specimen from Canchaque, Piura, Peru (FMNH-449321). ad- part of the anterior vas deferens penetrating at the phallus base; co - numerous fine raised threads that occupy almost all extension of the dorsal phallus; da- phallus location where the vas deferens opens; eb - expanded phallus base that forms two lateral expansions similar to brims; gd - distal extremity of the groove, often inclined on the phallus in direction of the shallow invagination in the ventral side; gr - longitudinal groove located on the left expanded brim of the phallus; in - shallow invagination delimited by the transversal ridge on the ventral side of the phallus, between the phallus base and its papilla; nr - ridges located near the distal part of the phallus, near the papilla; pa - papilla of the phallus; rm - phallus retractor muscle; sw- whitish swelling throughout the margin of the groove; tn - transversal ridge located between the base of the phallus and its papilla; ze - zig-zag raised threads located on each side of the groove edges. Scale = 2.5 mm.

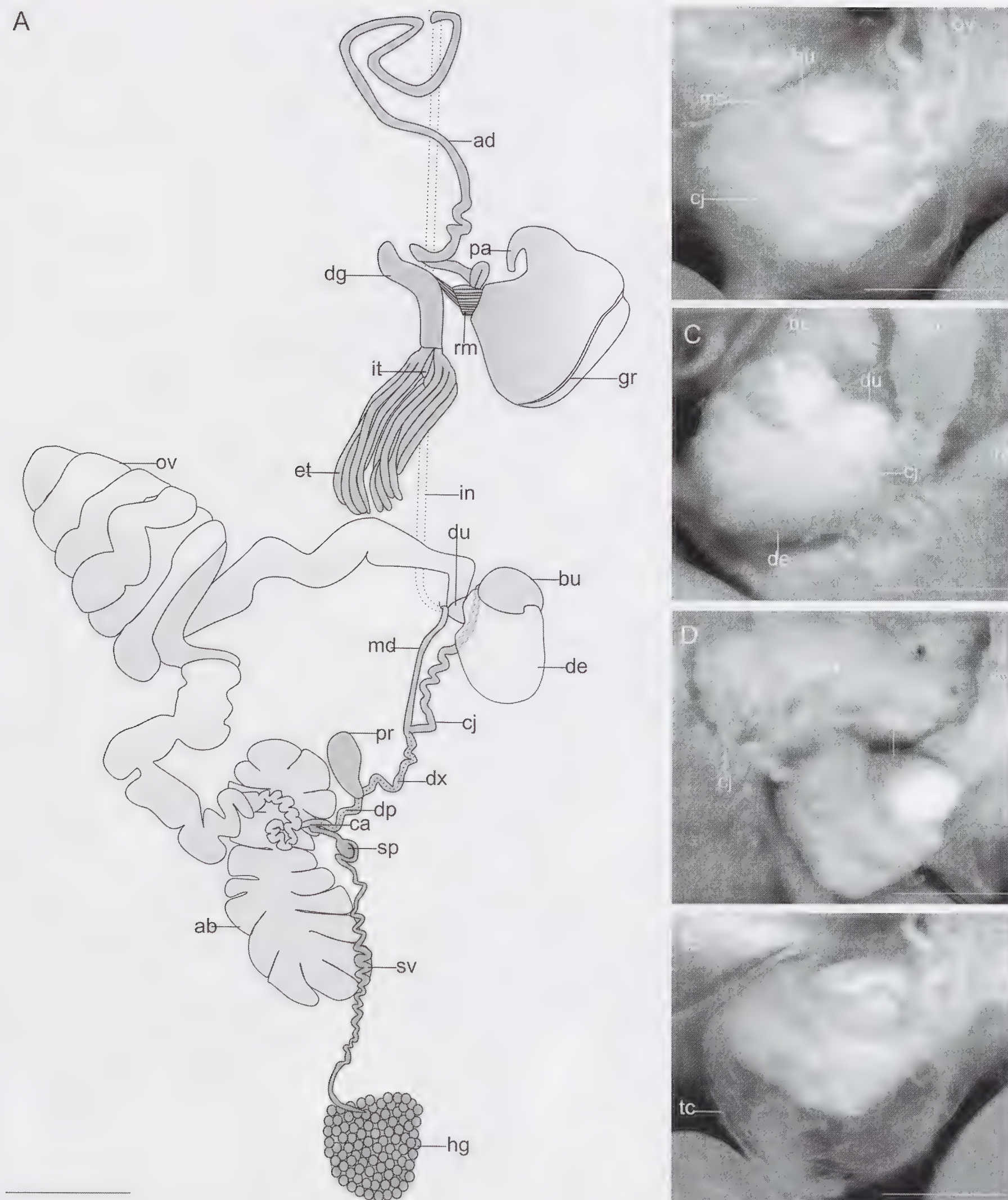


FIG. 4. Reproductive system in *Colosius confusus*, n. sp. A: Drawing of the reproductive system of a specimen intercepted from Colombia on July 15th, 2010 (USDA-1104452); B–E: Detail of the bursa copulatrix in specimen intercepted from Colombia on August 5th, 2010 (USDA-110447). B: Bursa copulatrix showing the canalis junctor strongly connected to the expanded bursa duct; C: Opposite view of B, showing the canalis junctor surrounding the bursa copulatrix duct before penetrating the bursa proper; D: Bursa copulatrix, after the canalis junctor and medium vas deferens disconnected from the duct bursa; E: Bursa copulatrix before the canalis junctor and medium vas deferens, evidencing the tissue that covers the bursa, medium vas deferens and canalis junctor. Ab - albumen gland; ad - anterior vas deferens; ca - carrefour region; bu - bursa copulatrix proper; cj - canalis junctor; de - expanded region of the bursa copulatrix duct; dg - papilla of the digitiform gland; dp - posterior distal vas deferens; du - region not expanded of the bursa copulatrix duct; dx - posterior proximal vas deferens; et - external and long tubules of the digitiform gland; gr - longitudinal phallus groove; hg - hermaphrodite gland; im - course of the vas deferens inside the tegument; it - internal and short tubules of the digitiform gland; md - medium vas deferens; ol - hermaphrodite duct; ov - oviduct; pa - papilla of the phallus; pr - prostate; rm - retractor muscle; sp - fertilization complex; sv - seminal vesicle; tc - tissue that cover the bursa copulatrix and duct around it. Scale = 2.5 mm.

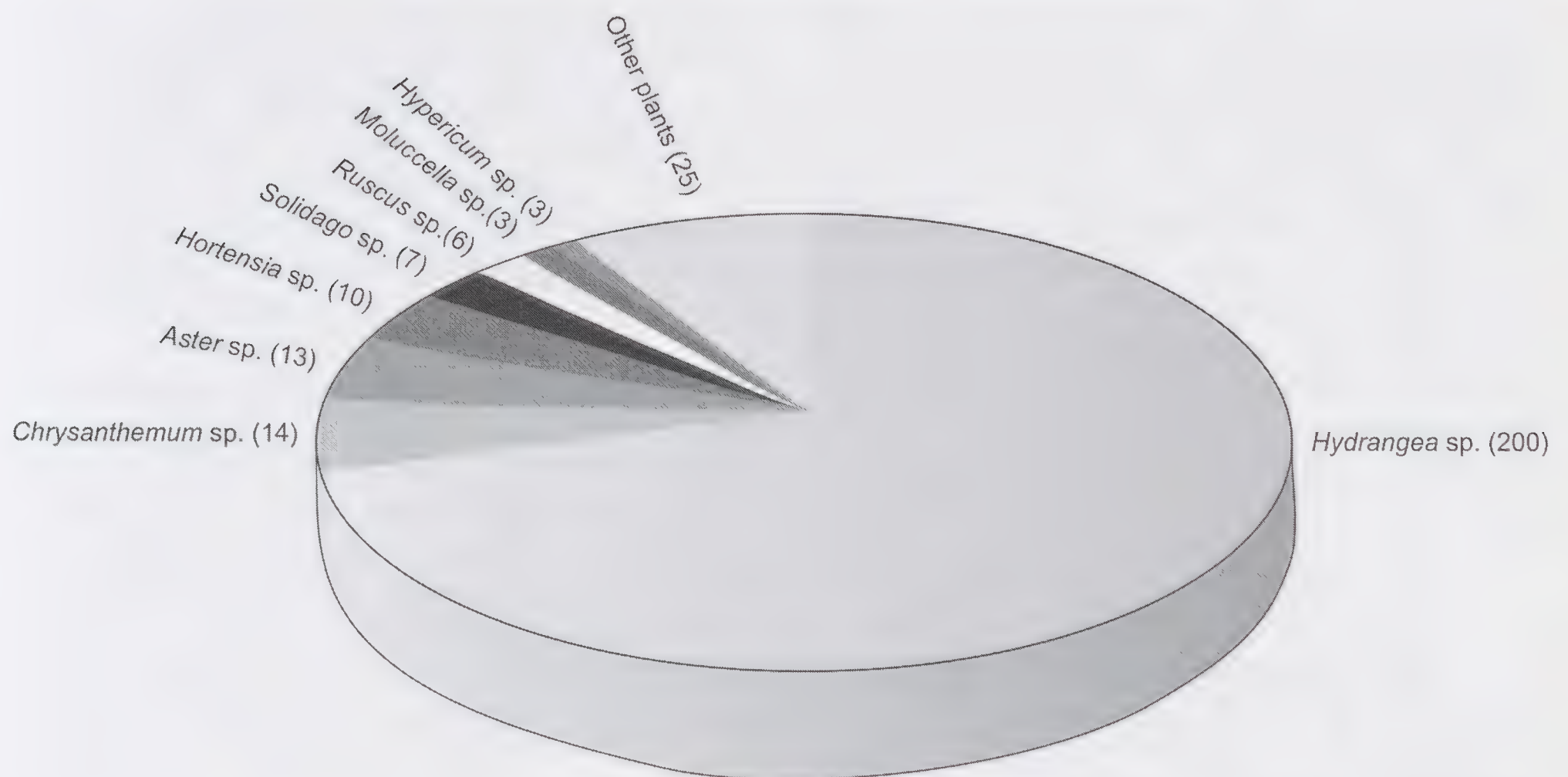


FIG. 5. Imports from the United States from 2001 to 2011, associated with interceptions of *Colosius confusus*, n. sp.

Internal Morphology

Pedal gland, salivary glands, pedal and pallial nerves and posterior pedal artery in *C. confusus*, n. sp., are morphologically similar to most species of Veronicellidae from Central and South America (Thomé, 1975). Diagnostic features are found in the reproductive system, in particular, bursa copulatrix and phallus.

Reproductive System (Figs. 3, 4, 13): All veronicellids are hermaphroditic and have an anteriorly located male genital pore and medially located female pore. A good description and illustration of the reproductive system in the family Veronicellidae is found in Thomé et al. (2006).

The phallus in *C. confusus*, n. sp., is very complex and variable (Fig. 3), assuming different conformations according to its distention, development level and the different folding. It is enveloped by a diaphanous muscular sheath (connected to the sheath of the papilla of the digitiform gland of same thickness, with which it shares a common atrium), and is strongly connected to the tegument by a short and thick retractor muscle. It is leaf-like with an expanded base, with two lateral brims and by a distally located papilla, where the vas deferens opens at its extremity. The right brim is more developed and has a deep longitudinal groove from its base near

the retractor muscle to close the papilla. A zigzag-shaped raised thread is present along the entire length of each side of the groove. The groove is often more expanded near the phallus base and distally near the papilla. On the dorsal part of the phallus, mainly from its $\frac{1}{2}$ distal, it is common a whitish swelling that is throughout the margin of the groove. A transversal ridge is present on the ventral side of the phallus, delimiting the base from the phallus papilla. This ridge also delimits a shallow invagination on the same side, which is variable in depth. The distal portion of the groove is often inclined on the phallus in the direction of the invagination. Numerous fine raised threads occupy nearly the entire extension of the opposite side of the phallus (dorsal side). A few ridges may also be evident next to the distal portion of the groove, near the papilla on the dorsal side. The papilla is conical and can vary in length, width, and proportion relative to the base.

Accessory to the phallus is the digitiform gland (analogous to the sarcobellum of some stylommatophorans, as described in Reise et al. (2011), which has tubules differentiated in two kinds: short internal tubules, and long external tubules that are very branched at the base and enclosed the shorter ones (Figs. 4A, 13). The internal tubules are generally lighter and thinner. In a sample of 40 specimens 1–3 internal tubules and 15–23 long



FIG. 6. Distribution of *Colosius confusus*, n. sp., Lighter red regions of Ecuador and Colombia correspond to regions of cut-flower production.

external tubules were found. The papilla of the digitiform gland is conical, narrow, long and tapered in the extremity.

In *C. confusus*, n. sp., after the medium deferens passes the prostate, which is pear-shaped and poorly developed (in general smaller than the bursa copulatrix), it continues free for a relatively long distance (Fig. 4A), before it penetrates in the tegument, near the duct base of the bursa copulatrix. The canalis junctor is long and sinuous and strongly compacted to the expanded bursa duct (Fig. 4B, D). In most specimens, the duct can seem to run inside the bursa duct. In younger specimens it is more easily separated from the bursa duct and more recognizable. The canalis junctor crosses attached to the bursa duct from its distal to the proximal region (near the female pore),

where it surrounds the right side of the duct, before it penetrates in the bursa copulatrix proper (Fig. 4C).

The bursa copulatrix is initially formed by a short non-expanded portion, close to the female genital pore. After, the duct is modified in a large lateral expansion that can be confused with the bursa proper, which is much smaller and has much thinner walls. The bursa duct internally has very developed pilasters that probably are responsible for expanding this region during mating, receiving and accommodating the developed lateral expansions of the phallus. Beyond this expansion there is a narrowing, which has width and length similar to that of the papilla of the phallus, which probably receives it. This portion is connected to the bursa proper, formed by a round sac with thin walls, where the sperm is

received and the excess probably destroyed (= gameolitic gland). The bursa appears to be laterally connected to the duct bursa, although it can be completely separate from the duct, after removing the tissue covering the bursa region (Fig. 4B, E). An accessory gland near the female genital pore is absent in *C. confusus*, n. sp.

Pedal Gland: The pedal gland, which is located on the anterior extremity of the sole under the head, is short and straight. Its posterior extremity is a little more expanded and is strongly connected to the pedal gland artery, at the height of the periesophagic nervous ring. In general, it is possible to distinguish two different areas in the pedal gland: an external lighter and an internal yellowish. The pedal gland opening is v-shaped.

Salivary Gland: Salivary gland is formed by large, individualized acini.

Pedal and Pallial Nerves: One pair of pallial and one pair of pedal nerves run parallel from the central nervous system towards the posterior region of the body. They are paired for a short distance, until around the pericardium height. From there, they diverge in an acute angle and, then run parallel and away from each other to the posterior region of the body.

Posterior Pedal Artery: The posterior pedal artery originates at the height of the central nervous system, running free between the pairs of pedal and pallial nerves for a short distance before penetrating the tegument, around the pericardium height.

Habitat

Specimens recently collected in Ecuador (USDA-110422, USDA-110424) were found in disturbed areas close to crops in Volcán Pululahua (Pichincha) and in an urban area, close to houses in Cantón Quijos (Napo), under rocks and wood. Specimens from Colombia and Ecuador intercepted were found in shipments of *Hydrangea* sp. (Fig. 5), but also with various other species such as *Chrysanthemum* sp., *Aster* sp., *Hortensia* sp., *Solidago* sp., *Ruscus* sp., *Moluccella* sp. and *Hypericum* sp. A few interceptions (two or less) also occurred in *Acacia* sp., *Alstroemeria* sp., *Eucalyptus* sp., *Gerbera* sp., *Gypsophila* sp., *Jatropha* sp., *Lisianthus* sp., *Mentha* sp., *Ornithogalum* sp., *Pittosporum* sp., *Polianthes* sp., *Rosmarinus*

spp. and *Veronica* sp. The species is recorded as *C. pulcher* causing damage to coffee crops in Colombia (Constantino et al., 2010). In Peru, the species was found attacking coffee in Canchaque, Piura. The animals use the base of plantain leaves to be hidden during the day, which is cultivated with coffee. In Amazonas, a different reddish-brown form was found living in urban areas, where other native species of slugs were also found, although not causing agriculture damage. Records of occurrence of this species, in the three countries, range from 1,500 m to 3,123 m of altitude.

Occurrence (Fig. 6)

Colombia: Caldas Department: Neira.

Ecuador: Imbabura Province: Caranqui, Ibarra; Pichincha Province: Volcán Pululahua; Napo Province: Cuyuja, Baeza, Cosanga, El Chaco, Cantón Quijos; Cotopaxi Province: Otonga; Tungurahua Province: Patate; Bolivar Province: San Miguel; Chimborazo Province: Alausí.

Peru: Piura Department: Canchaque; Amazonas Department: La Florida, Leymebamba; Cajamarca Department: Fazenda Monterezo.

Two specimens were intercepted from Ecuador. The others were intercepted from Colombia. Most of Colombia's cut flower production is located around Bogotá, with small production areas in the regions around Rionegro and Antioquia Medellin and Cali (USITC, 2003). In Ecuador, flower crops are concentrated in Pichincha (71%), Cotopaxi (22.2%), Imbabura (3.9%), Azuay (1.2%) and others (1.4%) (Acción Ecológica, 2000).

Genetic Variability

The genetic diversity within *C. confusus*, n. sp., is relatively low based on estimates using segments of 16S and COI mitochondrial DNA. This indicates that these regions could be useful for species diagnosis and delimiting species boundaries. The 28S, 16S, and COI genotypes sequenced from *C. confusus*, n. sp., samples are distinct from genotypes sequenced from *C. pulcher* and other species of *Colosius* studied, *C. lugubris* and *C. propinquus*. Based on phylogenetic analyses, *C. confusus*, n. sp., is a distinct lineage within the genus *Colosius* and not the sister taxon to *C. pulcher*. In comparison with *C. pulcher*, the *C. confusus*, n. sp., specimens can be identified based on unique nucleotide insertion and deletion events for 28S, 16S, and COI genes.

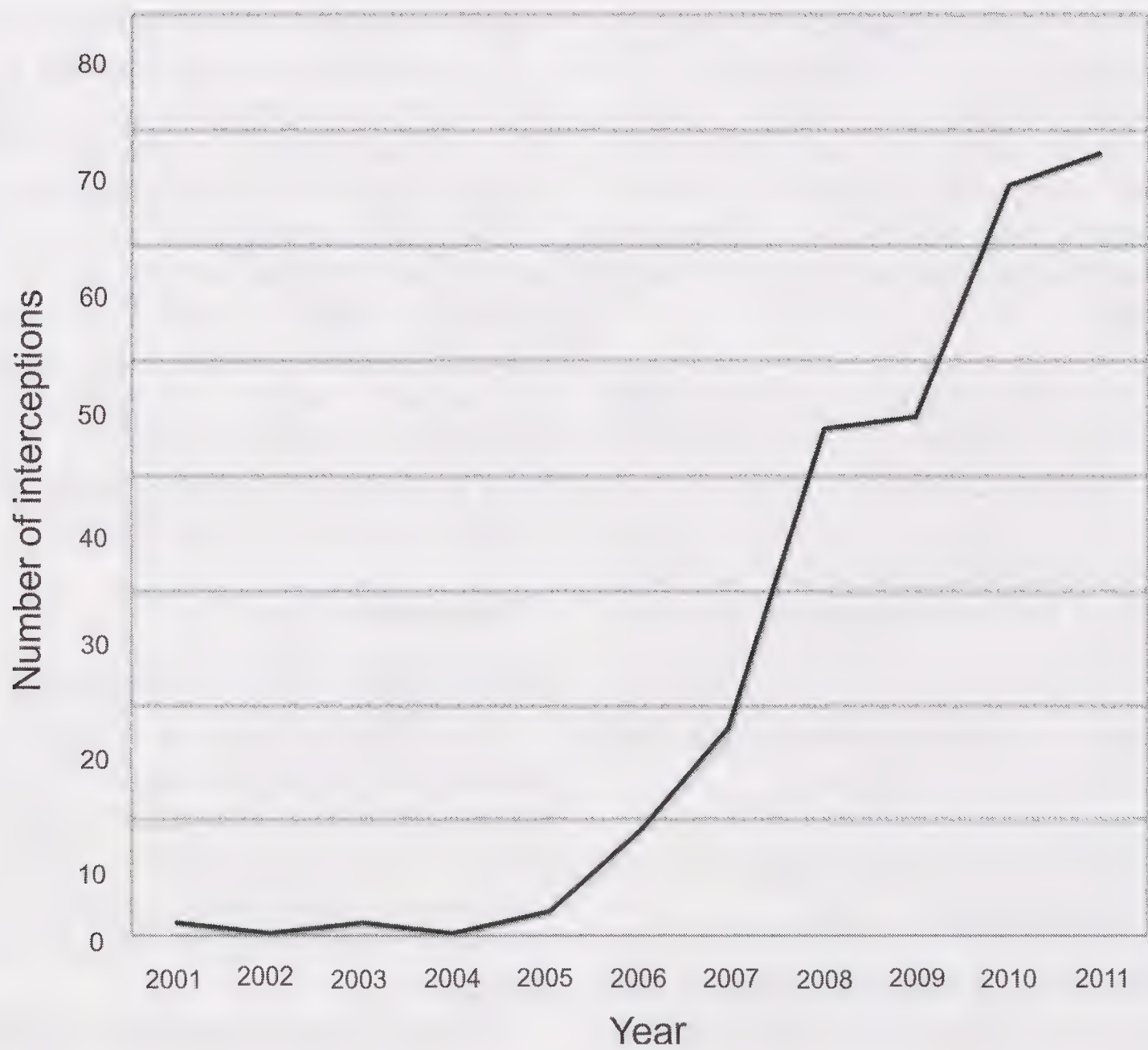


FIG. 7. Interceptions of *Colosius confusus*, n. sp., by year (2001–2011), by federal agricultural inspectors.

Remarks

The first record of *C. confusus*, n. sp., intercepted by federal agricultural inspectors was on April 6th, 2001 (USDA-110469), in Miami on *Gypsophila* sp. from Ecuador. In 2003, the species is intercepted for the first time from Colombia on *Moluccella* sp. In 2004 interceptions increased rapidly in imports from Colombia (Fig. 7), especially on *Hydrangea* sp. (Fig. 5).

Colosius pulcher (Colosi, 1921)
(Figs. 11–14)

Vaginula pulchra Colosi, 1921: 157.

Vaginula pulchra Colosi: Colosi, 1922: 496–498.

Vaginula pulchra Colosi: Thomé, 1970a: 23–25 (lectotype, paralectotype and type-locality designated).

Non Angustipes (Angustipes) pulcher of Colosi: Kraus, 1953: 63 (from Hacienda Taulis, Cajamarca Department, Peru).

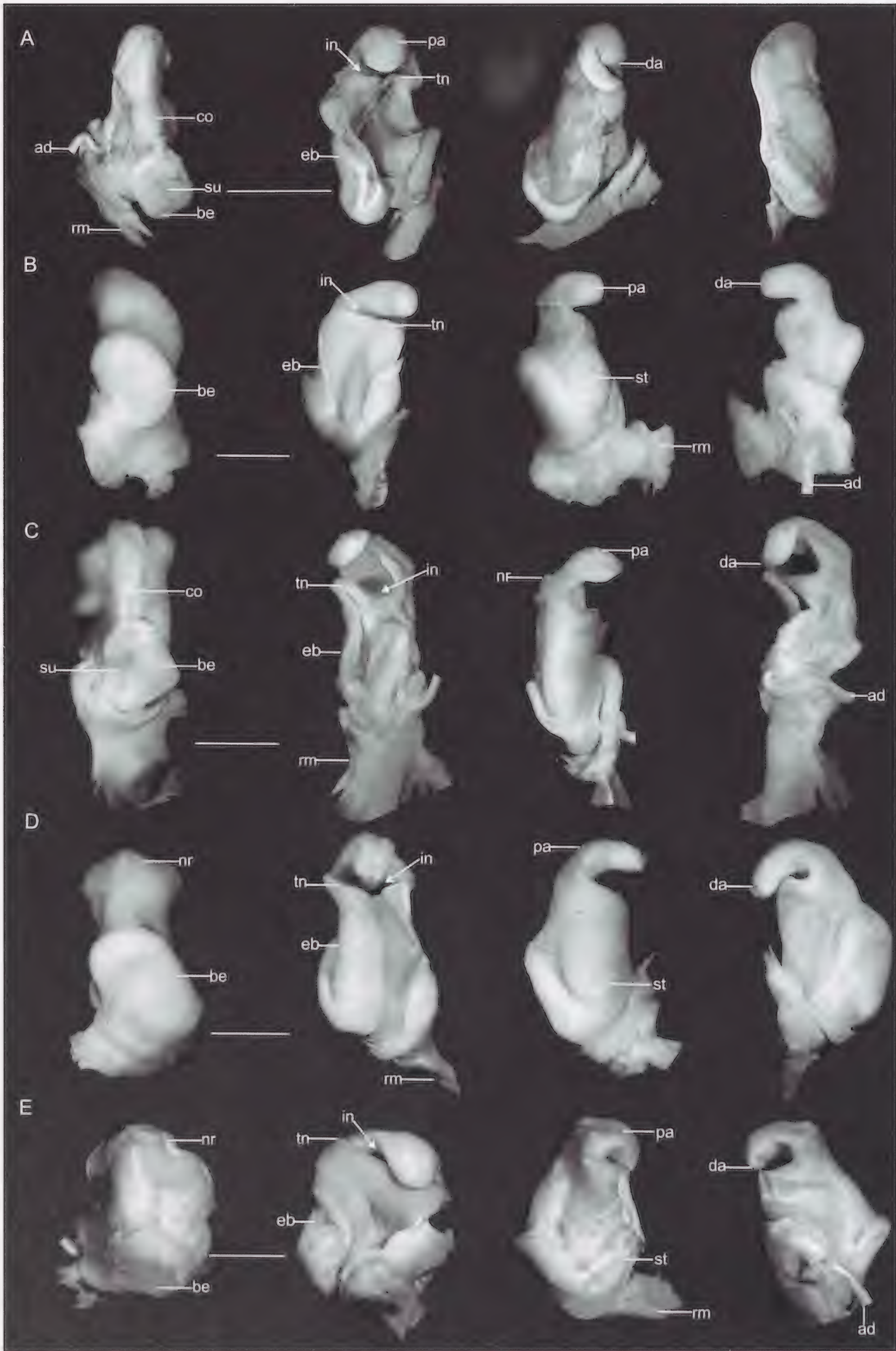
Colosius pulchrus: Thomé, 1975: 13; 1993: 72.

Non Colosius pulcher of Colosi: Thomé et al. 2001: 70–71 (from different localities from Peru).

Non Colosius pulcher of Colosi: Thomé et al. 1997: 522; ANSPA1092 (from “St. Domingo” – Dominican Republic).

FIG. 8. External coloration variation in adult specimens of *Colosius pulcher*. A: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110419B); B: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420B); C: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420C); D: Ventral view of the specimen shown in A (USDA-110419B); E: Ventral view of the specimen shown in B (USDA-110420B); F: Ventral view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420A); G: Ventral view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110421B); H: Dorsal view of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110419C); I: Dorsal view of the specimen shown in F (USDA-110420A). bs-black spots distributed on the entire notum; fp-female genital pore; fo-foot; yl- lighter line located longitudinally and centrally on the notum; ys-yellow spots that are distributed irregularly on the notum.





Colosius pulcher: Thomé et al., 1997: 522; USNM 769142.

Non Colosius pulcher of Colosi: Constantino et al., 2010: 1–8 (from Neira, Colombia).

Material Examined

QCZA-M-003647: Ecuador, Imbabura, San Pablo, N 00°15', W 078°10', 29.XII.1999. Col. G. Maldonado, 1 ex.; QCZA-M-003654: Ecuador, Imbabura, San Pablo, 19–20.I.2002., Col. F. Maza, 1 ex.; QCZA-M-003655: Ecuador, Imbabura, Lago San Pablo, 27.XII.1988, Col. U. Benítez, 1 ex.; QCZA-M-003662: Ecuador, Imbabura, Lita, 01.X.2001, Col. P. Villamar, 1 ex.; QCZA-M-003669: Ecuador, Imbabura, Peguche, 07.I.2001, Col. G. Buitrón, 1 ex.; QCZA-M-003672: Ecuador, Pichincha, Santo Domingo, 16.I.1993, Col. L. Torres, 1 ex.; QCZA-M-003676: Ecuador, Pichincha, Aloag, 1.XI.1983, Col. C. Padrez, 1 ex.; QCZA-M-003679: Ecuador, Pichincha, Santo Domingo, 4.XI.1983, Col. M. V. Gangotena, 2 ex.; QCZA-M-003697: Ecuador, Napo, Papallacta, 3,200 m, 8.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003698: Ecuador, Sucumbíos, Oyacachi, 3,200 m, 2.XI.1992, Col. L. Della Torre, 1 ex.; QCZA-M-003699: Ecuador, Napo, Papallacta, 3,200 m, 8.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003703: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 22.I.1989, Col. V. Nuñez, 10 ex.; QCZA-M-003706: Ecuador, Cotopaxi, Pujilí, XII.1993, Col. J. Arroyo, 1 ex.; QCZA-M-003707: Ecuador, Cotopaxi, Iliniza Sur, 21.I.1989, Col. F. Haro, 1 ex.; QCZA-M-003708: Ecuador, Cotopaxi, Boliche, 31.XII.1987, Col. M. Peñaherrera, 3 ex.; QCZA-M-003712: Ecuador, Cotopaxi, Iliniza Sur, 21.I.1989, Col. F. Haro, 1 ex.; QCZA-M-003713: Ecuador, Cotopaxi, Pastocalle, 3,900 m, 12.XII.1987, Col. M. Larrea, 1 ex.; QCZA-M-003714: Ecuador, Cotopaxi, Parque Nacional, 3,280 m, 04.XI.1995, Col. L. Torres,

1 ex.; QCZA-M-003718: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 12.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003719: Ecuador, Cotopaxi, Pastocalle, 3,406 m, 12.XI.1987, Col. M. Larrea, 3 ex.; QCZA-M-003720: Ecuador, Cotopaxi, Pastocalle, 3,400 m, 12.XII.1987, Col. M. Larrea, 2 ex.; QCZA-M-003721: Ecuador, Cotopaxi, Latacumba, IX.1981, Col. Gonore, 1 ex.; QCZA-M-003724: Ecuador, Cotopaxi, Latacumba, S 00°55'52", W 79°36'46", 2,770 m, 26.II.2000, Col. D. Alvarado, 1 ex.; QCZA-M-003728: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003729: Ecuador, Cotopaxi, Parque Nacional, 3,280 m, 04.IX.1995, Col. A. Muñoz, 1 ex.; QCZA-M-003733: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003735: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003738: Ecuador, Cotopaxi, Iliniza Sur, 3,400 m, 21.I.1989, Col. C. Ayala, 1 ex.; QCZA-M-003743: Ecuador, Chimborazo, Penipe-Puela, 2,300 m, 23.I.1986, Col. X.J. Jaurieth, 2 ex.; QCZA-M-003754: Ecuador, Carchi, San Gabriel, 27.XI.1989, Col. C. Puertas, 1 ex.; QCZA-M-003756: Ecuador, Carchi, San Gabriel, 27.IX.1989, Col. P. Enriquez, 1 ex.; QCZA-M-003762: Ecuador, Pichincha, Quito, 2,850 m, 02.I.1986, Col. C. Fierro, 1 ex.; QCZA-M-003763: Ecuador, Pichincha, Quito, 2,800 m, Col. Briones, 1 ex.; QCZA-M-003772: Ecuador, Pichincha, Quito, S 00°11'22", W 78°29'38", 20.XI.2000, Col. X. Haro, 1 ex.; QCZA-M-003774: Ecuador, Napo, Papallacta, S 00°22', W 78°09', 3,200 m, 17.I.1999, Col. S. Castelo, 1 ex.; QCZA-M-003776: Ecuador, Pichincha, Quito, S 00°11'22", W 78°29'38", 2,750 m, 05.I.2003, Col. D. Cevallos, 1 ex.; QCZA-M-003779: Ecuador, Pichincha, Pululahua, 15.IX.1989, Col. V. Perez, 1 ex.; QCZA-M-003782: Ecuador, Pichincha, Quito, 2,800 m, 04.I.1993, Col. G. Fletcher, 1 ex.; QCZA-M-003789: Ecuador, Pichincha, Quito,

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FIG. 9. Phallus variation in *Colosius pulcher*. Five different specimens (A–E) with the phallus in four distinct positions. The first position is the dorsal view, the second is the ventral view and the others are lateral views of the phallus. A: Specimen from Quito, collected by Dr. Festa (between 1895–1898), part of the material used by Colosi (1921) to propose *C. pulcher* (MRSN-3232); B: Specimen intercepted by USDA from Ecuador in 1969 in Miami (USNM-769142); C: Specimen from Mount Cotopaxi, Ecuador (FMNH-255555A); D: Specimen from Paschoa, Pichincha, Ecuador (USDA-110420D); E: Specimen from Quito (ZMUC, specimen A). ad - part of the anterior vas deferens penetrating the phallus base; be - oval to rectangular swelling found on the basal region of the phallus; co - longitudinal and central swelling often visible on the phallus; da - location on the phallus where the vas deferens opens; eb - phallus expanded base that forms two expansions similar to lateral brims; in - shallow invagination delimited by a transverse ridge on the ventral side of the phallus, between its base and papilla; pa - phallus papilla; rm - phallus retractor muscle; su - thin grooves can be seen on the basal swelling, similar to cracking; st - ridges that may be present on the left side of the expanded phallus base; tn - transversal ridge located between the base of the phallus and its papilla. Scale = 2.5 mm.

I.1983, Col. G. Paz, 1 ex.; QCZA-M-003790: Ecuador, Pichincha, Quito, 22.I.1990, Col. E. Guerrón, 1 ex.; QCZA-M-003791: Ecuador, Pichincha, Quito, 2,800 m, I.1986, Col. Brione, 1 ex.; QCZA-M-003792: Ecuador, Pichincha, Quito, S 0°9', W 78°26', 02.XI.2002, Col. A.V. Carvajal, 1 ex.; QCZA-M-003793: Ecuador, Pichincha, Quito, 2,800 m, 14.XI.1984, Col. L. Duque, 1 ex.; QCZA-M-003794: Ecuador, Pichincha, Quito, XII.1986, Col. G. Medina, 1 ex.; QCZA-M-003795: Ecuador, Pichincha, Calderon, 24.XI.1989, Col. L. Schel, 1 ex.; QCZA-M-003799: Ecuador, Pichincha, Pifo-Baeza, 1,900 m, 30.XI.1985, Col. A. Izurieta, 1 ex.; QCZA-M-003801: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Cordova, 5 ex.; QCZA-M-003802: Ecuador, Napo, Papallacta, 12.XII.1987, Col. A. Cordova, 3 ex.; QCZA-M-003803: Ecuador, Napo, Papallacta, 3,200 m, S 00°22', W 78°22', 17.I.1999, Col. S. Castelo, 1 ex.; QCZA-M-003804: Ecuador, Napo, Papallacta, 18.XII.1987, Col. A. Cordova, 1 ex.; QCZA-M-003805: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Córdoba, 4 ex.; QCZA-M-003806: Ecuador, Napo, Archidona, I.1987, Col. G. Medina, 1 ex.; QCZA-M-003807: Ecuador, Napo, Archidona, I.1987, Col. G. Medina, 1 ex.; QCZA-M-003808: Ecuador, Napo, Borja, 12.X.1990, Col. G. Aquilar, 1 ex.; QCZA-M-003809: Ecuador, Napo, Papallacta, 07.I.1989, Col. P. Coial, 1 ex.; QCZA-M-003810: Ecuador, Napo, Papallacta, 04.XII.1987, Col. A. Córdoba, 5 ex.; QCZA-M-003812: Ecuador, Napo, Papallacta, 13.XII.1987, Col. A. Córdoba, 1 ex.; QCZA-M-003814: Ecuador, Napo, Papallacta, 3,200 m, 08.I.1995, Col. K. Suárez, 1 ex.; QCZA-M-003818: Ecuador, Napo, Papallacta, 09.X.1987, Col. P. O. Jedo, 1 ex.; QCZA-M-003819: Ecuador, Napo, Papallacta, 25.I.1989, Col. C. Molina, 1 ex.; QCZA-M-003823: Ecuador, Napo, Papallacta, 07.I.1989, Col. P. Coral, 1 ex.; QCZA-M-003825: Ecuador, Napo, Papallacta, 3,159 m, 21.X.1995, Col. R. Paladines, 1 ex.; USDA-110419: Ecuador, Pichincha, Pasocha, S 00°25'12.38", W 078°31'11.64", 2,812 m, 19.IX.2011, Col. David et al., 3 ex.; USDA-110420: Ecuador, Pichincha, Pasocha, S 00°23'08.70", W 078°24'58.26", 2,736 m, 19.IX.2011, Col. D. G. Robinson et al., 30 ex.; USDA-110421: Hosteria Llacta Termale, Papallacta, Napo, Ecuador, S 00°21'46.08", W 078°08'55.68", 3,300 m, 20.IX.2011, Col. S. R. Gomes et al., 3 ex.; USDA-110423: Volcán Pululahua, Pichincha, Ecuador, N 00°02'16.43", W 078°30'01.56", 2,451 m, 21.IX.2011, Col. D. G. Robinson et al., 7 ex.; FMNH-255555: 1 mi. NE Mt. Cotopaxi, 2,956 m, 03.V.1970, Col. K. Campbell, 8 ex.; USNM-769142: Ecuador, USDA

interception on bromeliads, 18.Mar.1969, 1 ex.; MRSN-3232: Ecuador, Quito, 2,850 m, Col. Festa (paralectotypes), 3 ex.; ZMUC: Ecuador, Quito, Col. Banget, 8 ex.

Diagnosis (Fig. 9)

Species with a phallus formed by an expanded and muscular base that forms two lateral brims, and by a distal and conical papilla. Both (base and papilla) are delimited from each other by a transversal ridge on the ventral portion of the phallus, which also delimit a shallow invagination. An oval to rectangular swelling is present on the base on the dorsal side of the phallus. Ridges are also often on the right lateral expansion of the phallus.

External Morphology (Fig. 8)

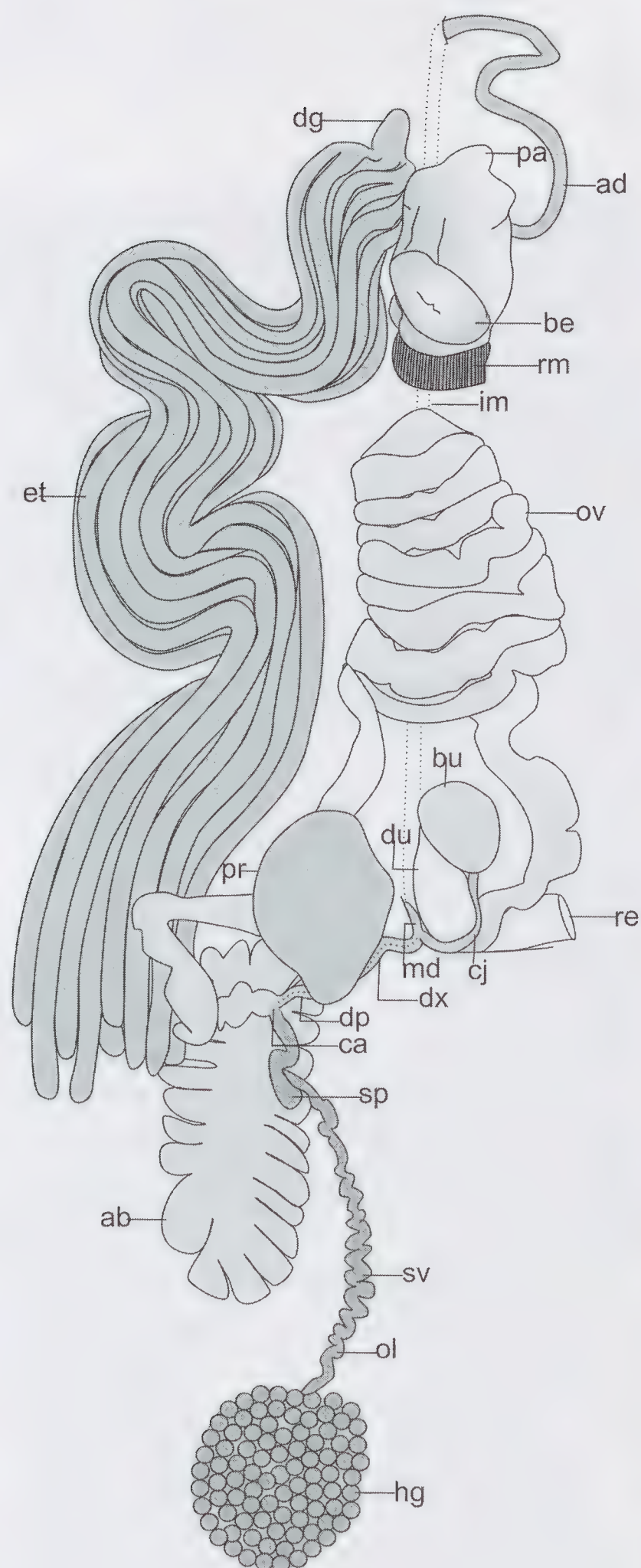
Adult specimens are of a medium size, with an extended length up to 8–9 cm. The notum and hyponota can have different shades of brown (Fig. 8A, B, G, H), ranging from dark (Fig. 8A) to light brown (Fig. 8B), but also yellowish-brown (Fig. 8C) and reddish-brown (Fig. 8I). Beige or yellow and black spots are found on this background. In general they are scattered over the entire notum without forming any specific pattern. Such spots may vary in quantity, defining a more homogenous coloration for the notum (Fig. 8A, I). A pale, thin line, located medially and longitudinally along the notum is almost always present, but it may also be absent or not continuous (Fig. 8A–C, I, M). Rarely two black bands are present on either side of the pale line. The coloration of the hyponota generally is the same as the notum background (Fig. 8D–F), but it can also be lighter. The hyponota often become paler immediately adjacent to the foot. The foot is light yellow and has approximately the same width as each hyponotum. The genital pore is located very close to the pedal groove (Fig. 8F). Ocular tentacles are bluish-gray or beige.

Internal Morphology

Pedal gland, salivary glands, pedal and pallial nerves and pedal artery have the same patterns described for *C. confusus*, n. sp.

A thin and translucent muscular sheath envelops the phallus of *C. pulcher*. The sheath is somewhat thicker than seen in *C. confusus*, n. sp. It is strongly connected to the tegument by a short, thick retractor muscle. It is leaf-like, having an expanded base that forms two

A



1 mm



FIG. 10. Reproductive system in *Colosius pulcher*. A: Drawing of the complete reproductive system of a specimen from Pasochoa, Pichincha, Ecuador (USDA-110420C); B–E: Detail of the bursa copulatrix in specimen from Papallacta, Napo, Ecuador (USDA-110421C). B: Bursa copulatrix prior to having the tissue around it, the canalis junctor and the medium vas deferens removed; C: Opposite view of bursa copulatrix shown in B, showing the canalis junctor surrounding the right side of the base of the bursa copulatrix duct before it penetrates the bursa proper, giving the wrong impression that it is penetrating the duct of the bursa; D: Bursa copulatrix and canalis junctor, after surrounding tissue has been removed, showing the penetration of the canalis junctor on the bursa proper; E: Bursa copulatrix region showing the spongy aspect of the tissue (shown compacted in picture B) located near the bursa copulatrix base. Ab - albumen gland; ad - anterior vas deferens; be - swelling found on the phallus base; ca - carrefour; cj - canalis junctor; bu - bursa copulatrix proper; du - bursa copulatrix duct; dg - papilla of the digitiform gland; dp - posterior distal vas deferens; dx - posterior proximal vas deferens; et - external and long tubules of the digitiform gland; im - vas deferens course inside the tegument; md - medial vas deferens; ol - hermaphrodite duct; ov - oviduct; pa - phallus papilla; pr - prostate gland; re - part of the rectum; rm - phallus retractor muscle; sp - fertilization complex; sv - seminal vesicle; hg - hermaphrodite gland (= gonad). Scale = 2.5 mm.



FIG. 11. Distribution of *Colosius pulcher*.

lateral brims on each side of the phallus, and a conical papilla, where at its distal extremity the vas deferens opens. The lateral expansions are less developed than those of *C. confusus*, n. sp., and are folded in the direction of the concave ventral side. On the ventral side there is a transversal ridge between the phallus base and its papilla, where there is a shallow invagination, which varies in depth between specimens. The papilla of the phallus is conic and short, less variable in length, width and proportion than seen in *C. confusus*, n. sp. It is often curved in the direction of the shallow invagination. On the dorsal side of the right expanded brim (opposite side of that where the vas deferens opens) ridges are commonly found, which range in degree of development. In addition, ridges may also be present at the boundary between the expanded base of the phallus and its papilla. In the basal region of the phallus there is an oval to rectangular swelling. Some thin grooves may be seen on this swelling, similar to cracking. In general, the phallus in *C. pulcher* is less variable than in *C. confusus*, n. sp. Variations are related to its width and degree of development and folding of their lateral brims. The shape, size

and exact position of the swelling found at the base of the phallus and degree of development of the ridges on the dorsum of the phallus are also variable.

The digitiform gland has internal short and external long and basally branched tubules as in *C. confusus*, n. sp. The papilla of the digitiform gland is conical and short. The digitiform gland is often longer than in *C. pulcher*. In a sample of 40 specimens, the external long tubules ranged from 17 to 24 and the short internal tubules from 5 to 12. The papilla is conical, short and triangular (Figs. 4, 10, 13).

The bursa copulatrix duct in *C. pulcher* (Fig. 10) has a medium length, is thick and has internally numerous pilasters, but it is not laterally expanded as in *C. confusus*, n. sp. The canalis junctor is long, but less sinuous and thicker than seen in *C. confusus*, n. sp. It is also strongly connected to the bursa copulatrix duct. After it runs connected on the entire length of the bursa duct, it surrounds the right base of the bursa duct before entering the base of the bursa copulatrix. The bursa proper is round to oval and composed of non-muscular, non-glandular tissue. Between the bursa duct and the rectum is a glandular structure that is

constituted internally by spongy compressed tissue (Fig. 10C, E). In *C. pulcher*, the prostate is more developed and the medial vas deferens is shorter than in *C. confusus*, n. sp.

Habitat

Specimens recently collected were found in areas near natural vegetation but also near

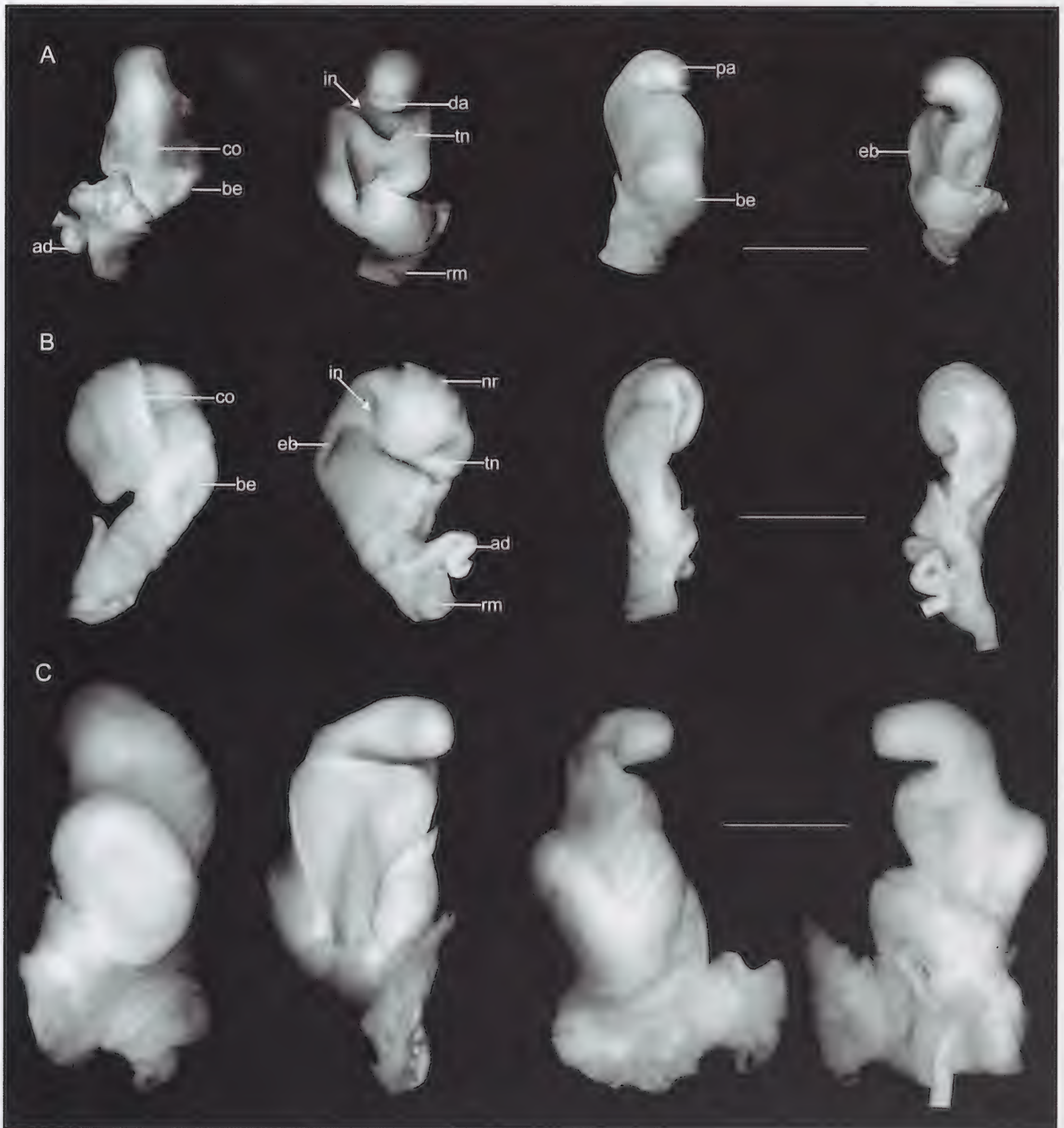


FIG. 12. Phallus of *Colosius propinquus* (A–B) and of *Colosius pulcher* (C) shown in the same proportion. A: Specimen from Reventador, Sucumbíos, Ecuador (QCAZ-M-003700); B: Specimen from El Angel, Imbabura, Ecuador (QCAZ-M-003659); C: Specimen intercepted by USDA in Miami from Ecuador in 1969 in Miami (USNM-769142). Ad - part of the anterior vas deferens penetrating in the phallus base; be - oval to rectangular swelling found on the basal region of the phallus; co - longitudinal and central swelling often visible on the phallus; da - location on the phallus where the vas deferens opens; eb - phallus expanded base that forms two expansions similar to lateral brims; In - shallow invagination delimited by a transversal ridge on the ventral side of the phallus, between its base and papilla; pa - phallus papilla; rm - phallus retractor muscle; tn - transversal ridge located between the base of the phallus and its papilla. Scale = 2.5 mm.

human habitation and in fields, as seen in the Volcán Pululahua in Paschoa, for example. During the day, they were found under rocks and woods. Most records of occurrence of *C. pulcher* are at high altitudes ranging from 1,229 to 3,400 m. Only two records are in low altitudes, which correspond to Santo Domingo (Pichincha) and Boliche (Cotopaxi), 479 m and 300 m above sea level, respectively.

Occurrence (Fig. 11)

Ecuador. Carchi Province: San Gabriel; Imbabura Province: Lita, Peguche, San Pablo; Pichincha Province: Aloag, Calderón, Volcán Pululahua, Pifo, Paschoa, Quito, Santo Domingo; Napo Province: Archidona, Borja, Oyacachi, Papallacta; Cotopaxi Province: Boliche, Iliniza Sur, Latacumba, Parque Nacional, Pastocalle, Pujilí; Chimborazo Province: Penipe; Cañar Province: Cañar (Colosi, 1921); Azuay Province: Cuenca, Gualaceo, Sigisig (Colosi, 1921).

Genetic Variability

Genetic variation within *C. pulcher* samples were measured using fragments of the 16S and COI mitochondrial genes. Based on small sample sizes (N = 5 for 16S and N = 8 for COI) genetic diversity was greater within *C. pulcher* than in *C. confusus*, n. sp. Although COI diversity was slightly greater in *C. pulcher* (0.3%) than in *C. confusus*, n. sp., (0.2%), diversity between two *C. pulcher* samples could be over 1% for 16S. This variation is still much lower than distances that separate species (i.e., > 4%). In the phylogenetic analysis *C. pulcher* was most closely related to *C. propinquus*. Although reddish and brown color-forms of this species have been found, our analysis of four brown and four reddish specimens using COI did not reveal evidence of genetic separation between color forms.

Remarks

There is only one record of *C. pulcher* intercepted by the USDA to date. This corresponds to a specimen examined by Thomé et al. (1997) and re-examined in this study, which had been intercepted on bromeliads from Ecuador on March 18th, 1969 (USNM 769142).

DISCUSSION

Only *C. lugubris* and *C. pulcher* were included with certainty in *Colosius* by Thomé (1975), while *C. propinquus*, *C. buergeri* and *C. festae* were included in the genus with reservations. The *C. propinquus* morphology is very similar to *C. pulcher*, but the phallus is less voluminous, as described by Colosi (1921, 1922) (Fig. 12). *Colosius propinquus* was considered an unidentified species by Thomé (1993) and a synonym of *C. pulcher* by Hoffmann (1925) and Kraus (1953). Two examined adult specimens from different localities (from El Angel, Imbabura; and from Reventador, both in Ecuador) near the type locality of *C. propinquus* (El Pun, Ecuador), match Colosi's morphological description (1921) for this species. Both specimens also showed the same haplotypes. Adult specimens of a form morphologically similar to *C. buergeri* (described originally based on juvenile specimens and which holotype is lost) were recently collected from Dominican Republic (Robinson et al., unpublished). This species, however, is related to species of Veronicellidae from Antilles, according to molecular data of a study in preparation by Robinson et al. Although the holotype of *C. festae* has been lost (Thomé, 1993), the phallus described by Colosi (1921, 1922) and Hoffmann (1925) provide sufficient information to distinguish it from *C. confusus*, n. sp., and from the other species of the genus. Although the monophyly of the genus is recovered in the trees, this relationship is not well supported by branch support values and is based on limited taxonomic sampling. Additional studies are required to formally test phylogenetic placements and monophyly of the genus.

Colosius confusus, n. sp., and *C. pulcher* can be externally indistinguishable, particularly when both have a dark brown notum (Figs. 2G, I, 8A). As in all species of the genus, these two species have a digitiform gland with very branched tubules at their base with a thick bursa copulatrix duct. Both species also have the digitiform gland with two kinds of tubules: internal short tubules and external long tubules, as seen in several other American genera (Thomé, 1975). Pedal gland, pedal and palial nerves, and the pedal artery are similar to several other American species of Veronicellidae (Thomé, 1975; Gomes & Thomé, 2004). In both species the phallus, considered the

principal diagnostic character in Veronicellidae, is leaf-like, with an expanded base that forms two lateral brims, and a conical papilla in their distal extremity. Base and papilla are delimited by a transversal ridge that borders a shallow invagination on the ventral side of the phallus (Figs. 3, 9, 12).

Despite sharing so many similarities, *C. confusus*, n. sp., and *C. pulcher* can be also differentiated by characteristics found on the phallus, digitiform gland and region of the bursa copulatrix. *Colosius confusus*, n. sp., has a deep longitudinal groove extending from the phallus base to its distal region, near the

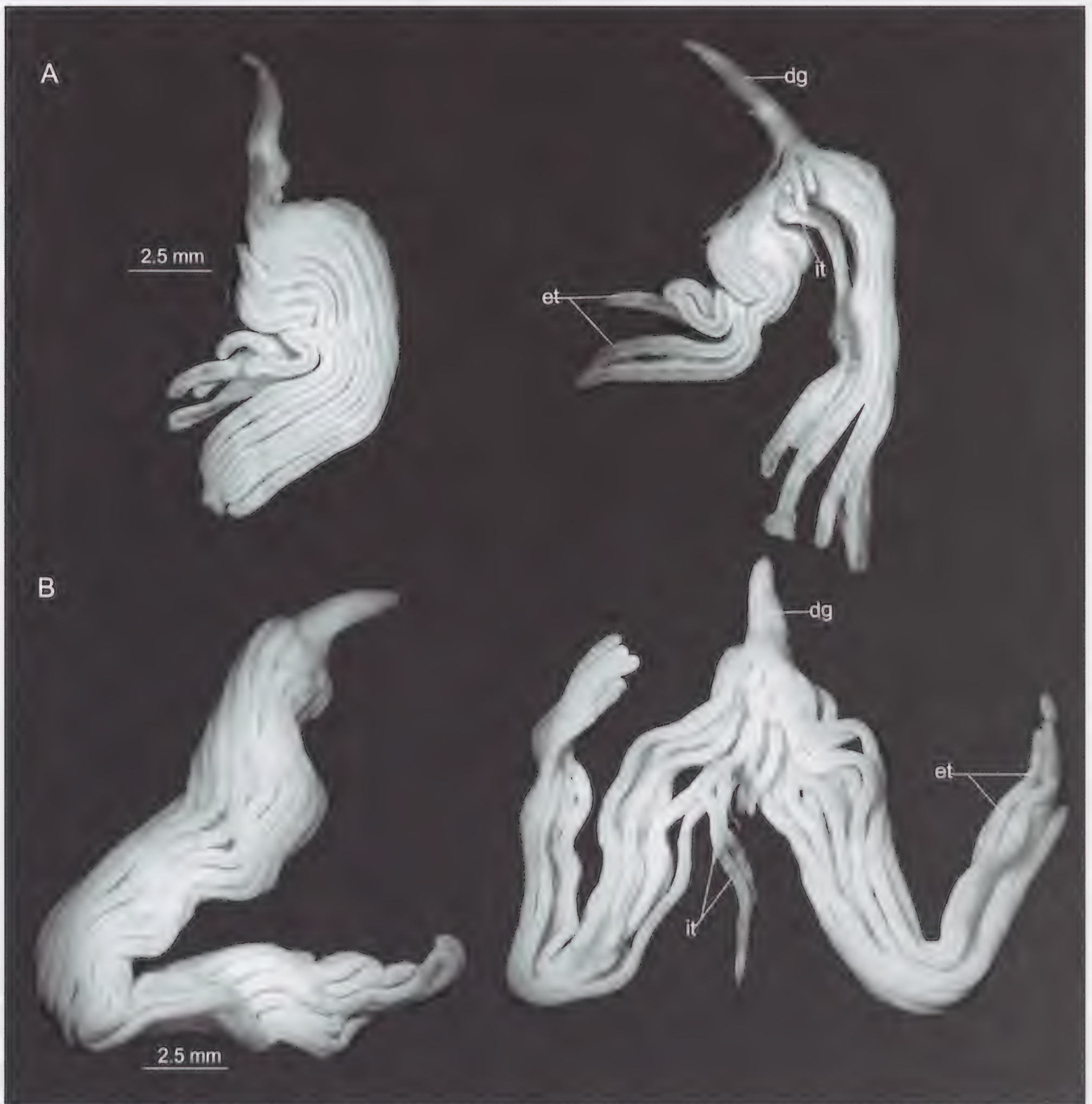


FIG. 13. Digitiform gland in a natural position and after the separation of the external tubules, to show the short internal tubules. A: Specimen of *Colosius confusus*, n. sp., in Miami on November 24th, 2007 (USDA-110451); B: *Colosius pulcher* from Volcán Pululahua, Pichincha, Ecuador (USDA-110423). Dg - papilla of the digitiform gland; et - long external tubules; it- short internal tubules.

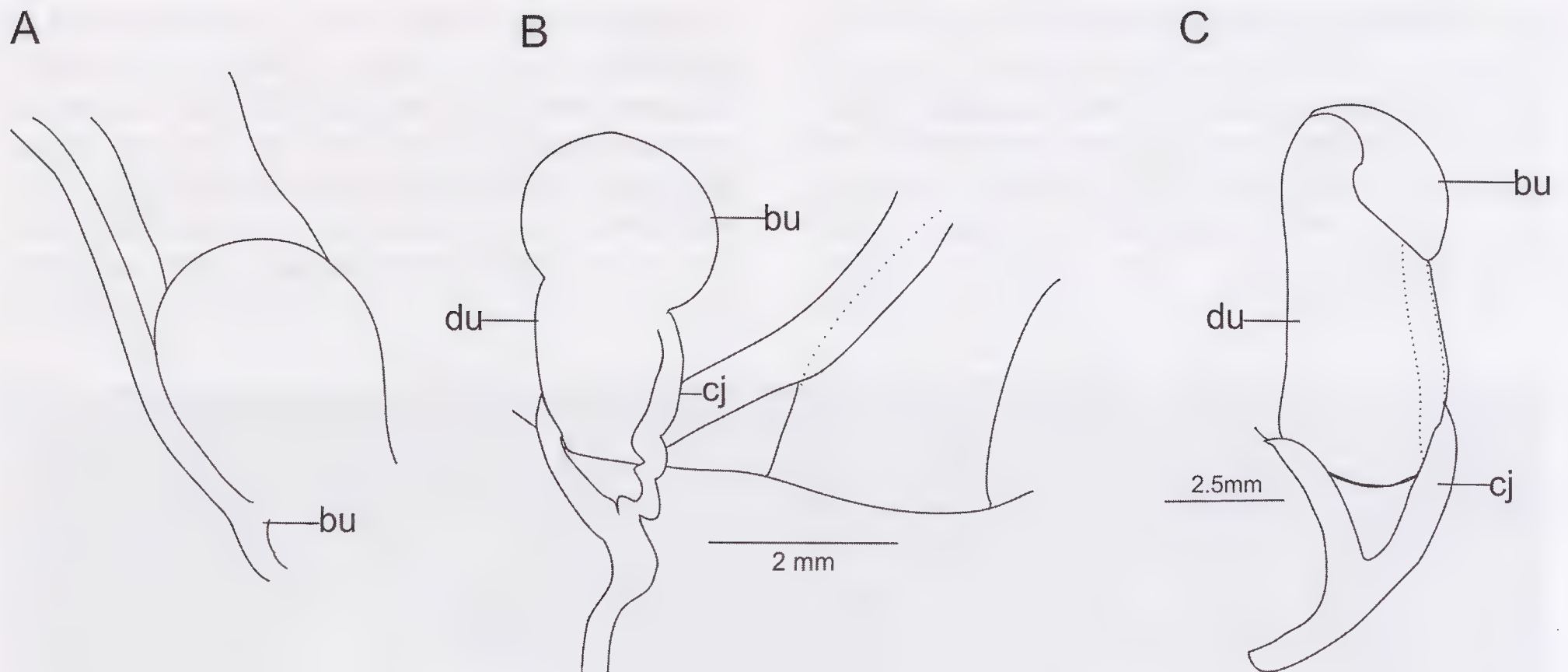


FIG. 14. Phallus illustration for *Colosius pulcher*. A: According to Colosi (1922); B: According to Thomé 1970b; C: The same position in ***Colosius confusus***, n. sp.

papilla. The dorsal side of the phallus is also covered by numerous fine raised threads. In *C. pulcher* the groove is absent and there is an oval to rectangular swelling at the basal region of the phallus (Figs. 3, 9). A few ridges are also often on the right lateral expansion of the phallus. In ***C. confusus***, n. sp., the internal short tubules range from 1 to 3 and in *C. pulcher* from 5 to 12 (Fig. 13). In juveniles these structures (groove and swelling) are also present on the phallus, although they are less developed. The number of tubules of the digitiform gland is also the same in young and adult specimens. The ridges on the phallus, however, are often absent in juvenile specimens. Both have a bursa copulatrix duct with a very thick duct with well-developed internal pilasters, but in ***C. confusus***, n. sp., the bursa duct is laterally expanded. The canalis junctor is also peculiarly long and sinuous in ***C. confusus***, n. sp. In addition, genetic differences in the DNA sequences of the species can be used to distinguish the species. Although not developed into a routine diagnostic method, the number of gaps and large divergence values between species provide useful information for confirming identification (Table 2). Genetic diversity within the 16S gene has been shown to be low within ***C. confusus***, n. sp., and *C. pulcher* and could serve as a practical diagnostic resource.

A leaf-like phallus with an expanded base and a distal papilla delimited from each other by a transversal ridge on the ventral side of the

phallus, where a shallow invagination can also be seen, so far described only for *C. pulcher* (and *C. propinquus*), additionally to the fact that *C. pulcher* was known only based on its original description and redescription of its lectotype and lectotypes, where only one similar phallus view was shown (Colosi, 1922; Hoffmann, 1925; Thomé, 1970b) (Fig. 14), as well the lack of studies about the Andean species of Veronicellidae were surely reasons that contributed to a series of mis-identifications involving *C. pulcher* (Thomé et al., 1997; Thomé et al., 2001; Constantino et al., 2010). Thomé et al. (1997) examined two specimens that he identified as *C. pulcher*, one from a USDA interception on bromeliads from Ecuador (USNM-769142) and another from the Dominican Republic (ANSP-A1092). The intercepted specimen is confirmed *C. pulcher* (Fig. 9B) and represents the only specimen intercepted to date, while the specimens from the Dominican Republic are a different species. This species is the same as the one recently found by Robinson et al. from the Dominican Republic (possibly an adult of *C. buergeri*). The species that Thomé et al. (2001) and Kraus (1953) called *C. pulcher* from Peru is an undescribed species, which will be described in a study in preparation, where the genus *Colosius* will be revised. The species that Constantino et al. (2010) called *C. pulcher* is ***C. confusus***, n. sp. (DPE CMS-1076), the new species proposed here. Thus, *C. pulcher* records, so far, are restricted to Ecuador (Fig. 11).

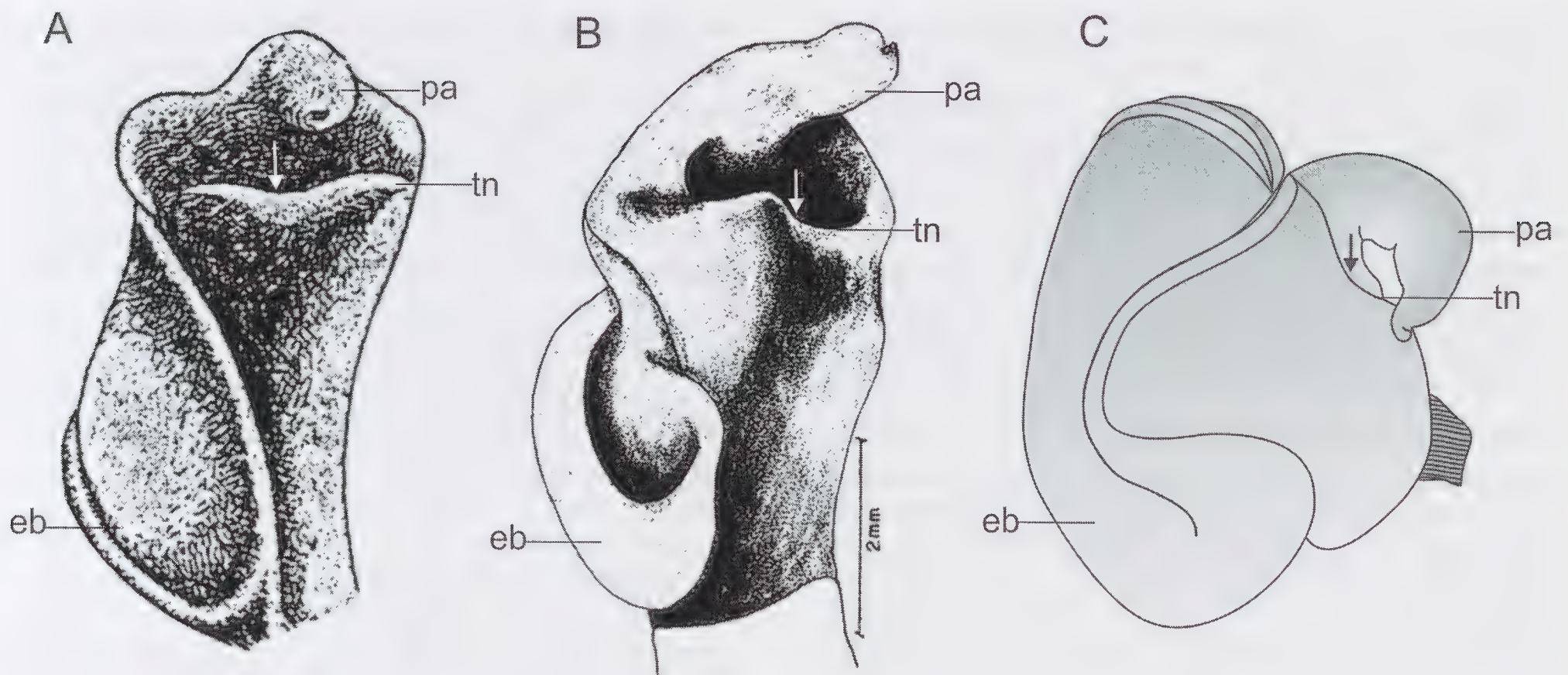


FIG. 15. Bursa copulatrix illustration for *Colosius pulcher*. A: According to Colosi (1922); B: According to Thomé (1970b); C: The same region in ***Colosius confusus***, n. sp.

Another issue involving *C. pulcher* is related to morphology of the bursa copulatrix. Colosi (1922) described it as being sessile with an extremely short duct where the canalis junctor penetrates. Thomé (1970b), based on specimens that he proposed as lectotype and paralectotypes, described the bursa copulatrix as formed by a very thick and well-developed duct, where the canalis junctor penetrates, near the bursa proper. According to our analysis, the bursa duct is well developed as described by Thomé (1970b) for the species, but it penetrates the bursa proper, instead of the bursa duct, different than that described for *C. pulcher* by these authors (Figs. 10, 15). An accessory gland is also present between the bursa duct and the rectum, in opposition of the description for this species held until now.

Colosius confusus, n. sp., was found in several locations in the Andean region of Ecuador in regions of flowers and coffee production in Colombia (Fig. 6). In Peru, it was found attacking coffee in Canchaque (northern Peru) and in two other Departments (Amazonas and Cajamarca). In Amazonas, it was found sympatrically with native species in urban areas, although not causing agricultural damage. The form found in Amazonas (2H, I) is also externally different from the forms found attacking coffee (Fig. 2A–G), although no genetic variation was found between them. The oldest specimen of the species in Peru was collected in 1970. In Ecuador, it was the second most commonly found species of Veronicellidae from

the Andean region, after *C. pulcher*, which is undoubtedly the most common one. Nevertheless, even after Colosi (1921, 1922) examined a large number of specimens from Ecuador, the author did not describe ***C. confusus***, n. sp. The first confirmed specimen of ***C. confusus***, n. sp., from Ecuador was collected in 1985 in Baeza, Napo province. Genetic information could aid in our understanding of ***C. confusus*** population structure, ancestral range, and dispersal patterns. The genes regions analyzed for our study, however, were selected to understand taxonomic differences. Although mitochondrial DNA (16S and COI) can provide information on the existence of isolated populations and evidence of migration, the diversity values measured within the species were low. This suggests that additional genes should be explored to study *Colosius* population genetics. The lack of variation observed in ***C. confusus***, n. sp., specimens from Ecuador, Colombia and Peru using mitochondrial DNA sequences is consistent with a recent migration/introduction between countries. Consequently, the large number of intercepted ***C. confusus***, n. sp., and material recently collected analyzed for 16S could not be traced back to a specific source population. ***Colosius confusus***, n. sp., was first intercepted by the USDA in 2001 on *Gypsophila* sp. from Ecuador. Only since 2003 has there been a significant increase of ***C. confusus***, n. sp.'s interceptions from Colombia (Fig. 7), mainly on *Hydrangea* sp., even though the United States has imported

aproximately 80% of Colombian flowers during the period 1997–2001 (USITC, 2003). The damage to coffee crops in Colombia seems secondary. Only recently, Constantino et al. (2010) reported the species (as *C. pulcher*) causing damage to coffee plantations in Colombia, in the municipality of Neira (Caldas), at 1,700 m altitude. A populational study based on a large number of specimens of *C. confusus*, n. sp., from a wide geographic area would be needed to determine its origin, but our results give some indications that the species is likely to be exotic to both Ecuador and Colombia, and may originally be from Peru. In Ecuador, the production of cut flowers began in the late 1970s with exports in the early 1980s (Acción Eco, 2000). The spread of *C. confusus*, n. sp., could be related to expanding commercial flower production.

The continuous and rapid increase of interceptions of *C. confusus*, n. sp., by agricultural inspectors in the United States supports the emergence of a increasingly important agricultural pest that is still uncontrolled, with a potential ability to adapt and become a pest of different kinds of crops, with a great potential of being spread due to its close association with the cut-flowers industry. It offers thus significant economic risk, which also poses an environmental risk including be competing with native species.

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PATTERNS OF LAND SNAIL ASSEMBLAGES ALONG A FINE-SCALE MOISTURE GRADIENT

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ABSTRACT

We studied changes of terrestrial snail assemblages over a gradient of soil moisture using 60 sampling plots in the White Carpathian Mountains of the south-eastern Czech Republic. We used within-site design to control for confounding effects of site characteristics other than humidity, and we directly measured soil moisture along nine transects at distinct locations. Each transect had from 4 to 16 plots, and it was laid down from wet calcium-rich spring fen habitats to semi-dry meadows in the fen surroundings. We observed a sharp moisture gradient along each transect, with the measured soil moisture varying from 97% in fen plots to 19% in semi-dry grassland plots. Altogether 29 land snail species and 4,213 live individuals were collected. Species richness of land snails varied from 2 to 11 species per plot. However, we did not observe any significant linear or unimodal response of species richness or total abundances to measured soil moisture. In contrast, sharp compositional changes along studied transects were found, suggesting differences in species preference to soil moisture conditions at fine, within-site scale. Among 21 species with the frequency higher than five, 10 (after a Bonferroni correction) showed a significant response to soil moisture. Three species expressed significant affinity to drier plots, five species were more abundant in moister plots and only two species preferred middle values. In several previous studies, both linear and hump-shaped relation between soil moisture and number of land snail species were documented. This raised questions about general response of land snails to soil moisture and the importance of possible bias caused either by using only estimated values of site moisture or sampling in distinct sites differing also in other environmental factors that might potentially overtopped importance of soil moisture for land snail distribution.

Key words: calcareous fens, grasslands, molluscs, soil moisture, species optima, species richness, vegetation.

INTRODUCTION

Many previous studies on land snail community ecology have shown a positive response of species richness and mostly also total abundances to content of available calcium and/or soil pH (e.g., Wäreborn, 1969; Horsák, 2006). This pattern seems to operate at both larger (regional) and smaller (within-site) scales (Juříčková et al., 2008). The amount of calcium also dictates changes in species composition, with the majority of species having their optima at calcium-rich sites (Hylander et al., 2005; Horsák et al., 2007), but Nekola (2010) found many strictly acidophilous species in a North American fauna. Soil moisture is the second most frequently, but notably

less often than calcium, reported environmental predictor of land snail distribution (e.g., Martin & Sommer, 2004a, b), because snails are sensitive to desiccation (Cook, 2001). However, contrary to calcium content and soil pH, soil moisture has rarely been directly measured (but see Wäreborn, 1969). It was mostly only estimated (Getz & Uetz, 1994; Chiba, 2007) or studied sites were classified into several moisture categories (Martin & Sommer, 2004a, b). Some studies used Ellenberg indicator values (Ellenberg et al., 1992) as a proxy for soil moisture obtained from vegetation samples (Čejka et al., 2008; Čejka & Hamerlík, 2009). These estimates were found to perform well in land snail ecology studies (Horsák et al., 2007).

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However, all previous studies assessing the influence of soil moisture on land snail distribution have analysed assemblages of isolated and distant sites, mostly at relatively large, regional sampling scale. This sampling protocol may introduce bias, caused by other site characteristics independent of soil moisture, which can influence land snail distribution as well.

Confounding effects of soil moisture and calcium content were already documented in several studies. Wäreborn (1969) found a positive effect of soil moisture only at dry meadow wood series, but no effect of calcium. In wet meadow wood series, however, the amount of calcium was closely related with the snail species richness. Martin & Sommer (2004b) found no effect of soil pH in dry forest sites, but a positive response was observed at damper forest habitats.

In this study, we attempt to avoid the drawbacks of previous studies and to analyse land snail assemblage patterns along a steep soil-moisture gradient along a transect from a treeless calcareous-fen community to a dry calcium-rich meadow. To obtain more general results, we settled transects at nine distinct locations, but within a climatically and geologically uniform area. Our aims were to study response of snail species richness, total abundance and composition along a fine-scale gradient of soil moisture. We also analysed the pattern of species composition changes along the gradient – species turnover or their accumulation toward plots of certain soil moisture values (i.e., higher, middle or lower) – using species response curves. On the basis of above-referred published data, it is difficult to predict whether soil moisture controls both species richness and species composition or only one of them. If there is a significant effect on the species richness, we can predict two possible responses that have been reported in the literature – a linear decrease towards drier sites (Wäreborn, 1969; Martin & Sommer, 2004a, b) or a hump-shaped response, with a peak at intermediate moisture values (Getz & Uetz, 1994; Chiba, 2007).

METHODS

Study Area

The study area is situated in the southern part of the border between the Czech and Slovak republics in the White Carpathian

Mountains and adjacent southern part of the Vsetínské Mountains, a part of the western Carpathians. This area is formed by flysch, the bedrock composed of alternating clay stones and sandstones (Poprawa & Nemčok, 1988). Annual mean precipitation is approximately 700 mm and annual mean temperature is about 7.5°C (Miklós, 2002; Tolasz, 2007). Localities were situated between 270 and 550 m a.s.l. Vegetation consisted of both meadow calcareous tufa-forming spring fens classified as the *Caricion davallianae* alliance (cf. Hájek et al., 2006) and of semi-dry grasslands classified as *Festuco-Brometea* class (alliance *Cirsio-Brachypodium pinnati* or *Bromion erecti*). There were also ecotonal communities, classified within *Calthion* alliance, located between these two vegetation types in some locations. Nomenclature of vegetation follows Chytrý (2007). The geological structure, climatic conditions and vegetation of the study area are in detail described by Horsák (2006) and Jongepierová (2008). Studied sites were *a priori* chosen to represent as long as possible within-site gradient of soil moisture and vegetation structure (Schamp et al., 2011). We investigated places where both moist spring fen and dry grasslands occurred alongside. Snail fauna of treeless spring fens was found to belong to the most species-rich snail assemblages of the region (Dvořáková et al., 2011), holding the highest number of species among grassland habitats (Horsák, 2008).

Field Sampling and Environmental Variables

Data were collected in June and July during the years 2005–2008. At each of nine study locations, one linear transect (7–31 meters) was laid out from the moistest part of spring fen to the dry grassland surrounding the fen. The number of plots varied from 4 to 16 per transect, depending on the size of spring fen. The size of each plot was 75 cm x 75 cm and the distance between centres of two neighbouring plots was two meters. In total, 60 plots were sampled.

To sample mollusc assemblages, a central nested sub-plot (25 cm x 25 cm) of each plot was cut just below ground level, using a sharp knife, and removed along with the herbaceous vegetation, mosses, litter and the upper soil layer. To obtain more precise information about the entire assemblages of the plot, we also randomly collected 3 litres of the upper layer from the remaining part of sampled plot. Plots were not cut completely to reduce negative

TABLE 1. List of all land snail species recorded based on live individuals in all study plots. Frequency (Freq.) is a number of plots with the species occurrence, density (Dens.) is total number of live individuals recorded. Results of species abundance response curve modelling (GAM) for soil moisture (Opt.; species optimum in dry plots = D, wet plots = W, in the middle of the moisture gradient = M) and the model significance (P) are given, x = no candidate additive model had AIC value lower than the null model. Significant correlations after a Bonferroni correction are in bold ($P < 0.00238$).

Abbrev.	Species name	Freq.	Dens.	F	P	Opt.
PunPyg	<i>Punctum pygmaeum</i> (Draparnaud, 1801)	39	651	x	x	-
CocLub	<i>Cochlicopa lubrica</i> (O. F. Müller, 1774)	38	598	2.84	0.050	-
VerPyg	<i>Vertigo pygmaea</i> (Draparnaud, 1801)	37	544	47.90	< 0.001	W
TruCyl	<i>Truncatellina cylindrica</i> (A. Férussac, 1807)	21	1026	11.15	0.001	D
VerAng	<i>Vertigo angustior</i> Jeffreys, 1830	21	174	2.24	0.094	-
PerHam	<i>Perpolita hammonis</i> (Ström, 1765)	20	138	5.67	0.020	D
VerAnt	<i>Vertigo antivertigo</i> (Draparnaud, 1801)	20	185	19.55	< 0.001	W
VitPel	<i>Vitrina pellucida</i> (O. F. Müller, 1774)	17	202	46.20	< 0.001	D
CarMin	<i>Carychium minimum</i> O. F. Müller, 1774	14	75	3.62	0.019	M
PliLub	<i>Plicuteria lubomirskii</i> (Ślósarski, 1881)	13	15	4.66	0.034	D
EuoStr	<i>Euomphalia strigella</i> (Draparnaud, 1801)	11	17	3.25	0.030	M
ValPul	<i>Vallonia pulchella</i> (O. F. Müller, 1774)	10	53	1.84	0.151	-
EucFul	<i>Euconulus fulvus</i> (O. F. Müller, 1774)	9	14	11.97	< 0.001	W
SucPut	<i>Succinea putris</i> (Linné, 1758)	9	56	11.26	< 0.001	W
ValCos	<i>Vallonia costata</i> (O. F. Müller, 1774)	9	213	9.00	< 0.001	M
VitCon	<i>Vitrea contracta</i> (Westerlund, 1871)	9	12	6.03	0.017	D
CocLul	<i>Cochlicopa lubricella</i> (Rossmässler, 1835)	8	25	20.11	< 0.001	D
VerMou	<i>Vertigo moulinsiana</i> (Dupuy, 1849)	8	26	9.11	< 0.001	M
SucObl	<i>Succinella oblonga</i> (Draparnaud, 1801)	7	43	6.77	0.001	W
AcaAcu	<i>Acanthinula aculeata</i> (O. F. Müller, 1774)	6	51	2.40	0.079	-
VerSub	<i>Vertigo substriata</i> (Jeffreys, 1833)	6	41	3.12	0.035	M
TroHis	<i>Trochulus hispidus</i> (Linné, 1758)	5	7	not tested (because of low frequency)		
CarTri	<i>Carychium tridentatum</i> (Risso, 1826)	4	28			
CepVin	<i>Cepaea vindobonensis</i> (A. Férussac, 1821)	4	9			
VitDia	<i>Vitrea diaphana</i> (Studer, 1820)	3	3			
ColEde	<i>Columella edentula</i> (Draparnaud, 1805)	2	3			
AegMin	<i>Aegopinella minor</i> (Stabile, 1864)	1	1			
AriArb	<i>Arianta arbustorum</i> (Linné, 1758)	1	1			
OxyEle	<i>Oxyloma elegans</i> (Risso, 1826)	1	2			

impact of the sampling on fen communities. Samples from fen habitat were processed using the “wet sieving method” (Horsák, 2003) and then air dried. Samples from grasslands were dried immediately after the sampling. After drying, shells were separated from the remaining material by hand sorting under a stereo microscope, identified and counted, separating live individuals and empty shells. We analysed only live individuals, because

the calcium rich environment of study sites supports shell accumulation over a relatively long time period (Cernohorsky et al., 2010). This can introduce a significant bias in the data, as empty shells of species inhabiting adjacent habitats can frequently be found. Because the sampling was designed to extract snail individuals from sampling volume, live individuals show an exact snail assemblage of the plots; we did not recorded any important seasonal

variation in species' occurrences at fens in the study region (M. Horsák, unpubl. data). We also carried out all analyses based on all recorded shells (live individuals and empty shells together), and we obtained basically the same results as presented below (data not shown). This suggests that the observed patterns are stable over the season. Mollusc nomenclature follows Horsák et al. (2010), with authorities given in Table 1. Slugs were not included in this study, as the sampling method used was not suitable to determine slug abundance (cf. Oggier et al., 1998). Moreover, there is only one species, *Deroceras laeve* (O. F. Müller, 1774), which occupies spring fens more frequently; occurrence of further slug species is mostly accidental.

Moisture in the upper layer of the soil horizon was measured on each plot during dry period of the year (July to September) using a moisture meter (ThetaProbe, soil moisture sensor ML2x). To gather more information about environmental conditions in each plot, cover of vascular plants, mosses, and all plants together were estimated. Samples of vascular plant standing biomass and upper soil layer were taken from each nested sub-plot just before the sampling of mollusc assemblages. Concentration of nitrogen and calcium were subsequently analyzed from dried and weighed plant biomass; volume of calcium was determined also from soil samples. Before chemical analyses, the plant biomass samples were air-dried at 70°C. For nitrogen determination, dry material was mineralised with sulphuric acid and hydrogen peroxide, and the nitrogen concentration determined by the distilling method using a Kjeltex apparatus. For determination of calcium, material was mineralised in a sealed system, using microwave heating. Calcium concentration was determined by atomic absorption spectrophotometry (Zbiral, 1994). Plant-available calcium in the soil was extracted using the Mehlich III method (strong acid extraction with ion complex) and determined by atomic absorption spectrophotometry (Zbiral, 1995). Soil pH was not used in the analyses, because a range of values was too narrow (pH_{H2O} 6.2–8.0), as base-rich soils occurred at all plots, and no pH effect was found in preliminary analyses (data not shown).

Data Analysis

Altogether 29 land snail species, 353 of their records and 4,213 live individuals were

collected. For comparison of species richness between plots, two variables were introduced: (1) species richness, represented by a count of all species in a target plot and (2) proportional representation of snail species, calculated by dividing number of species found in a target plot by all species found in the target locality. Relationships between variables representing species richness and number of individuals on one hand and all measured variables on the other were statistically explored by Spearman's rank correlation coefficient in STATISTICA 10 program (Statsoft, Inc., 2011). A Bonferroni correction was used to remove the problem with multiple testing.

To find the main ecological gradients in snail species data, we used detrended correspondence analysis (DCA; Hill & Gauch, 1980) in CANOCO 4.5 program (ter Braak & Šmilauer, 2002). Species abundance of snails were log-transformed as $Y = \log_{10}(n+1)$ before the ordination analyses to reduce the influence of dominant species and to extract the pattern based mainly on presence/absence data, as some changes in population sizes can occur during the season. The environmental variables with significant relationship with ordination axes for snail species composition were determined using Spearman's rank correlation coefficient. Relationship between number of species and plot scores on the first DCA axis was explored by the Generalised Linear Models (GLM; McCullagh & Nelder, 1989), with Poisson distribution function, calculated in R program (version 2.12.2; R Core Team, 2012). Significances of linear and quadratic response were compared by Chi-square test.

Snail species response curves were modelled for species with more than five occurrences in the dataset. Non-transformed species abundance (density) was used to explain the distribution of species along the moisture gradient. We applied Generalised Additive Models (GAM; Hastie & Tibshirani, 1990) with Poisson distribution, as implemented in CanoDraw for Windows 4.0 program (ter Braak & Šmilauer, 2002). Selection of the best fitted regression model was done manually based on Akaike information criterion (AIC; Akaike, 1974), and its significance was tested using F-test. Three categories of response curve shapes were subjectively distinguished: (1) an increasing monotonic response towards the dry part of moisture gradient or (2) towards the wet part, and (3) a unimodal response curve with optimum in the central part of the gradient.

TABLE 2. Descriptive statistic of all variables.

Variables	Minimum	Lower quartile	Mean	Median	Upper quartile	Maximum	Standard deviation
Soil moisture (%)	19	27	61	71	88	97	29
Ca-soil (mg/g)	3.1	13.3	24.4	25.3	36.3	40.7	12.1
N-biomass (%)	0.95	1.19	1.3	1.32	1.41	1.67	0.17
Ca-biomass (%)	0.53	0.79	0.96	0.92	1.08	1.97	0.28
Biomass weight (g)	7.2	13.4	22.3	20.7	27.9	52.1	9.9
Total cover (%)	55	73	84	88	93	100	12
Herb cover (%)	30	60	72	70	80	100	14
Moos cover (%)	0	1	44	40	90	95	39
Nr. of species per plot	2	4	6	5	8	11	2
Nr. of species per site	7	10	14	15	17	17	4
Nr. of live individuals per plot	2	22	70	44	74	922	122

RESULTS

There was a sharp moisture gradient along each transect, with the measured soil moisture varying from 97% in fen plots to 19% in plots located in the surrounding semi-dry grasslands. Soil moisture and calcium content were the most variable factors along the transects, with most of the other measured variables strongly correlating with them (Tables 2, 3). Soil calcium content was extremely high in all samples (Table 2).

Towards wet and calcareous plots in central parts of fens, the percentage cover of moss layer notably increased, whereas herb cover,

related with weight of biomass, decreased. The decrease was however somewhat weaker, which resulted in an increase of total plant cover (moss and herb cover together) with soil moisture (Table 3).

Species richness of land snail assemblages varied from 2 to 11 species per plot. Although we expected rather tight response of species richness to soil moisture gradient, only a weak and negative correlation was found (Fig. 1, Table 4). After a Bonferroni correction, only two significant correlations remained – proportional representation of species richness was positively correlated with weight of dry biomass and with cover of herb layer (both these vari-

TABLE 3. Values of Spearman’s rank correlation coefficient between environmental variables and their significance. Significant correlations after a Bonferroni correction are in bold ($P < 0.00625$).

Variables	Soil moisture		Ca-soil		N-biomass		Ca-biomass		Biomass weight		Total cover		Herb cover	
	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P	r_s	P
Ca-soil	0.66	<0.001												
N-biomass	0.03	0.837	-0.22	0.090										
Ca-biomass	0.21	0.116	0.11	0.384	-0.08	0.569								
Biomass weight	-0.45	<0.001	-0.38	0.002	-0.15	0.270	-0.22	0.092						
Total cover	0.43	<0.001	0.43	<0.001	-0.19	0.147	0.22	0.095	0.09	0.492				
Herb cover	-0.46	<0.001	-0.42	<0.001	0.15	0.251	-0.11	0.396	0.70	<0.001	0.25	0.051		
Moss cover	0.82	<0.001	0.75	<0.001	-0.24	0.069	0.27	0.036	-0.32	0.012	0.68	<0.001	-0.31	0.013

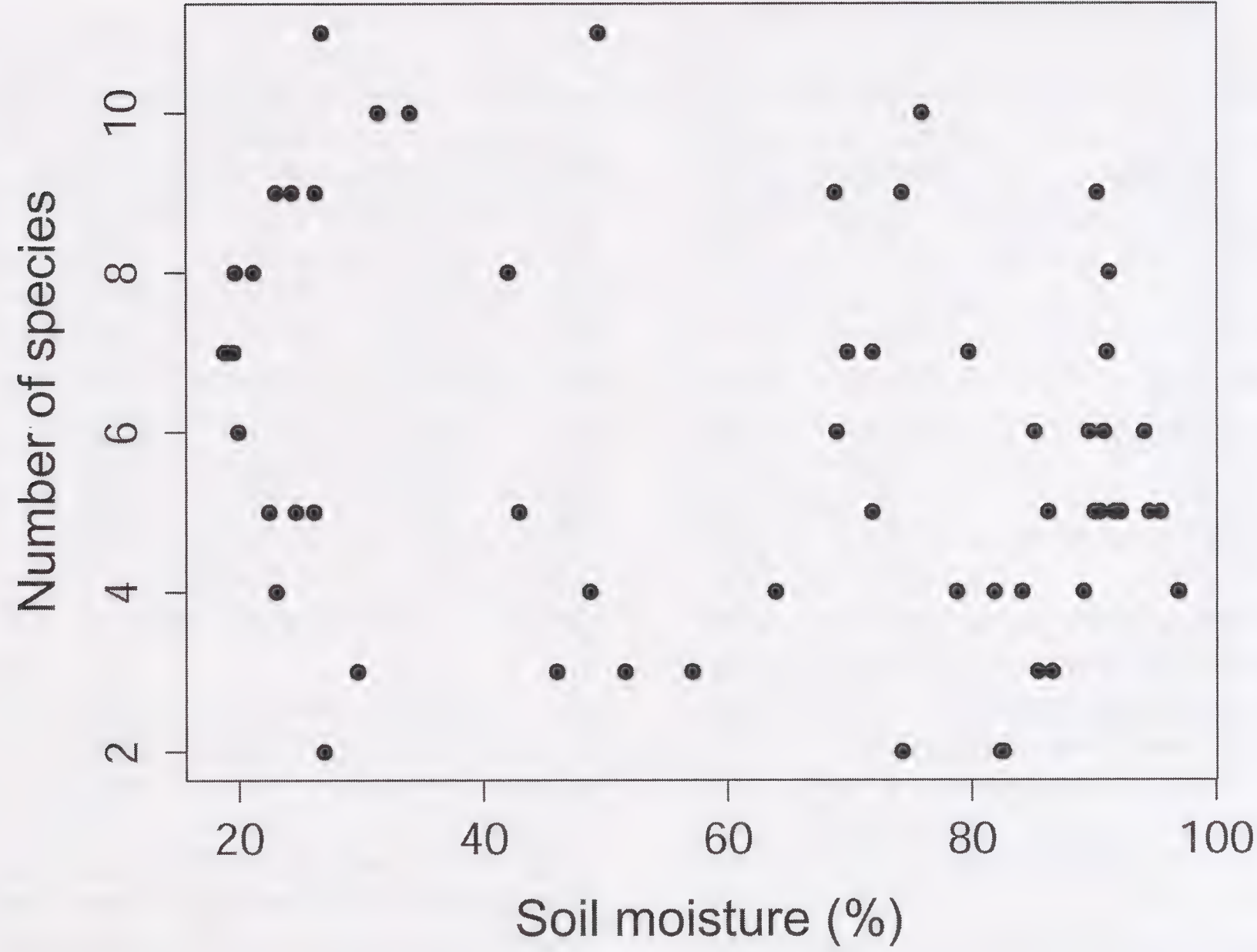


FIG. 1. Scatter plot between soil moisture and species richness.

ables were closely related; Table 3). Correlations between both species richness and total abundances and the other variables were not significant (Table 4). We also did not find any non-linear relationship between soil moisture and any measure of species richness and total

abundances (data not shown). In contrast, land snail species composition tightly responded to soil moisture gradient, with soil moisture having the highest fit to the first DCA axes (Fig. 2; Table 5). We also found a significant hump-shaped relationship (Chi-square statistic: 59.49 on 2

TABLE 4. Values of Spearman’s rank correlation coefficient between land snail species richness and all environmental variables, and their significance. For detail description see Methods. Significant correlations after a Bonferroni correction are in bold ($P < 0.00625$).

Variables	Species richness		Species proportional representation		Number of individuals	
	r_s	P	r_s	P	r_s	P
Soil moisture	-0.28	0.031	-0.24	0.069	-0.08	0.561
Ca-soil	0.09	0.501	0.06	0.639	0.21	0.100
N-biomass	-0.26	0.045	0.05	0.722	-0.24	0.060
Ca-biomass	-0.12	0.363	-0.07	0.595	0.28	0.033
Biomass weight	0.34	0.008	0.38	0.003	0.22	0.094
Total cover	0.12	0.344	0.08	0.560	0.06	0.660
Herb cover	0.30	0.018	0.40	0.001	0.05	0.705
Moss cover	-0.17	0.188	-0.22	0.098	-0.01	0.948

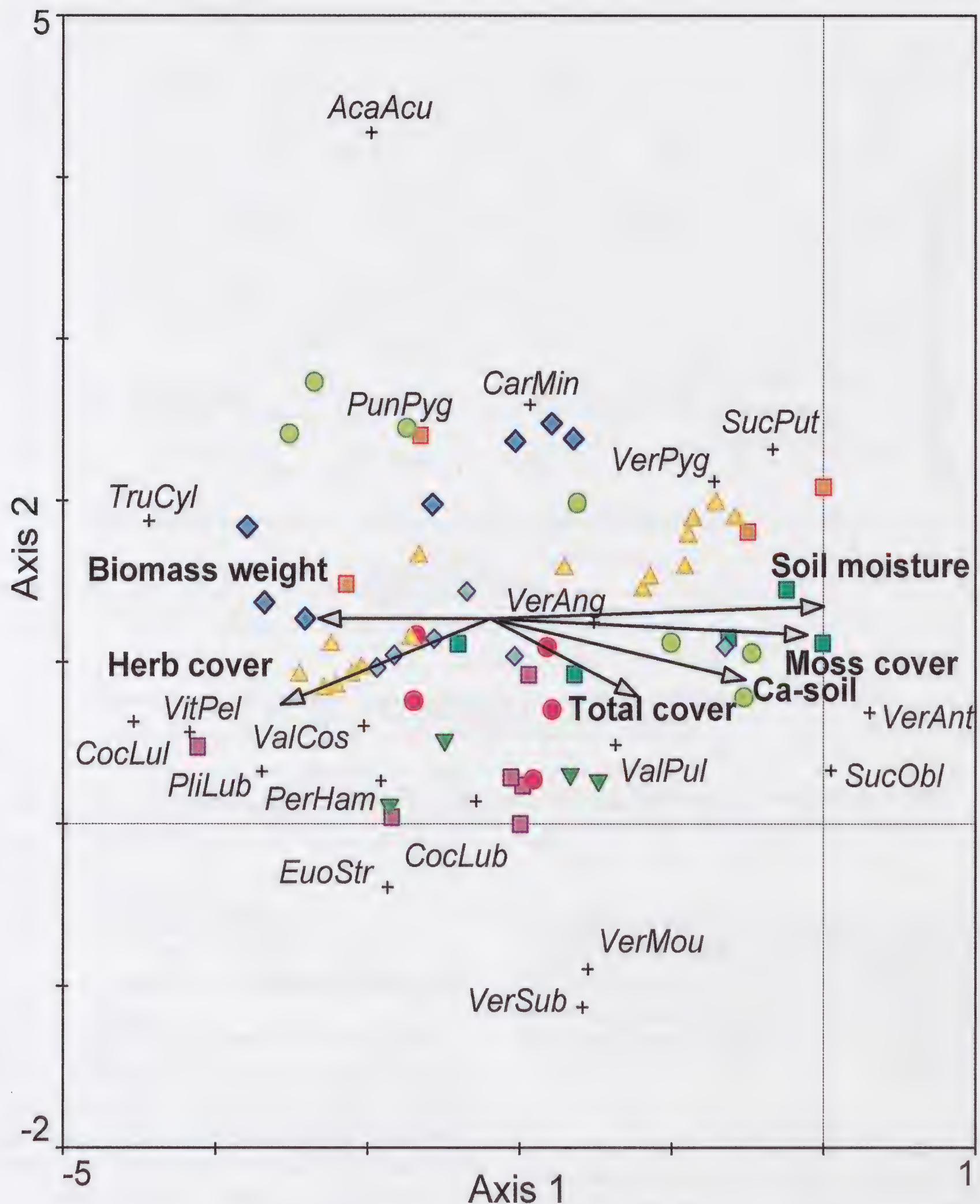


FIG. 2. Detrended Correspondence Analysis (DCA) of land snail species data with the plot scores on the first two ordination axes. Type of symbols represents individual transects. Species with the higher fit (more than 10%) and only significant variables ($P < 0.01$) were plotted. Eigenvalues: 1st axis = 0.528, 2nd axis = 0.311; explained variation: 1st axis = 15.2%, 2nd axis = 9.0%. For full species names see Table 1.

and 57 DF, approximate $r^2 = 0.273$, $P < 0.002$) between sample scores on the first DCA axes and species richness (Fig. 3).

Among 21 species with the frequency higher than five, 16 showed a significant response to soil moisture; 10 were significant also after a Bonferroni correction (Table 1). Six species expressed affinity to drier plots (i.e., *Cochlicopa lubricella*, *Perpolita hammonis*, *Plicuteria*

lubomirskii, *Truncatellina cylindrica*, *Vitrea contracta* and *Vitrina pellucida*), five species were more abundant in moister plots (i.e., *Euconulus fulvus*, *Succinea putris*, *Succinella oblonga*, *Vertigo antivertigo* and *V. pygmaea*), and another five species (i.e., *Carychium minimum*, *Euomphalia strigella*, *Vallonia costata*, *Vertigo moulinsiana* and *V. substriata*) expressed a unimodal response with the preference to

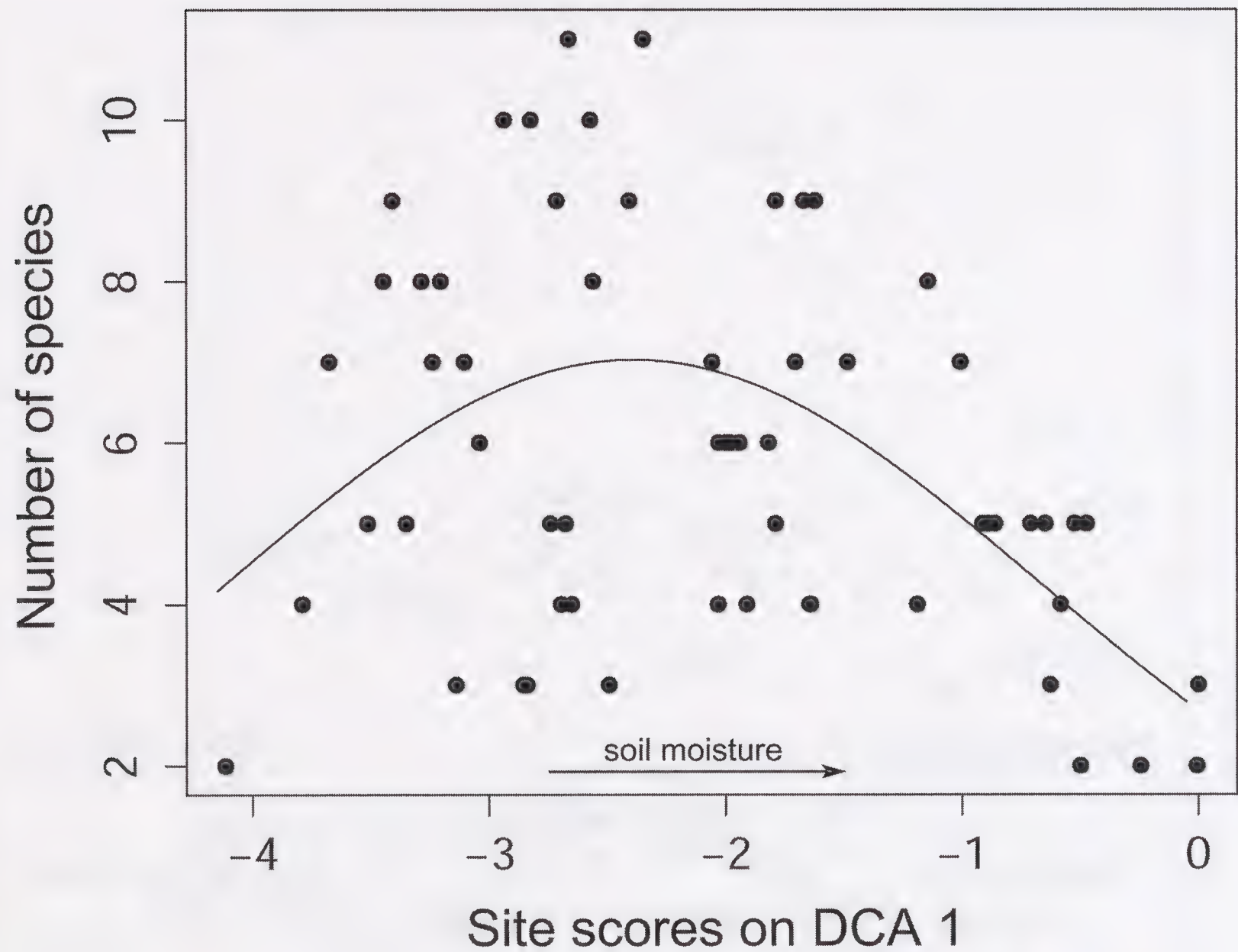


FIG. 3. Relationship between plot scores on the first DCA axis of snail samples and species richness (GLM, approximate $r^2 = 0.273$, $P < 0.002$).

intermediate moisture conditions. Several frequent species, such as *Cochlicopa lubrica*, *Punctum pygmaeum* and *Vertigo angustior*, occurred evenly along the whole moisture gradient resulting in a non-significant response (Fig. 4, Table 1).

TABLE 5. Values of Spearman’s rank correlation coefficient between environmental variables and the first two DCA axes, and their significance. Significant correlations after a Bonferroni correction are in bold ($P < 0.00625$).

Variables	AX1		AX2	
	r_s	P	r_s	P
Soil moisture	0.81	<0.001	0.16	0.210
Ca-soil	0.64	<0.001	0.03	0.821
N-biomass	0.16	0.218	-0.11	0.420
Ca-biomass	0.13	0.315	-0.12	0.375
Biomass weight	-0.45	<0.001	-0.01	0.957
Total cover	0.32	0.013	-0.15	0.255
Herb cover	-0.58	<0.001	-0.32	0.013
Moss cover	0.76	<0.001	0.02	0.873

DISCUSSION

Species Richness

Although both species richness and total abundance varied considerably among the plots (Table 2), we have not found any significant relationship between these two variables and soil moisture when species richness was corrected for a site’s species pool (Table 4). Aboveground biomass and herb-layer cover were the only significant predictors of proportional species richness, suggesting certain role of vegetation as a shelter and food source for snails (Cook, 2001; Dvořáková & Horsák, 2012).

Several previous studies exploring effects of soil moisture on land snail species richness documented a positive response, with increasing species richness and/or abundances towards wetter habitats (e.g., Wäreborn, 1969; Martin & Sommer, 2004a, b). However, it seems that these patterns are site or system specific, related probably also to compound effects of the other environmental variables important for snails, such as soil pH and calcium content in topsoil. For example, Martin & Sommer (2004b)

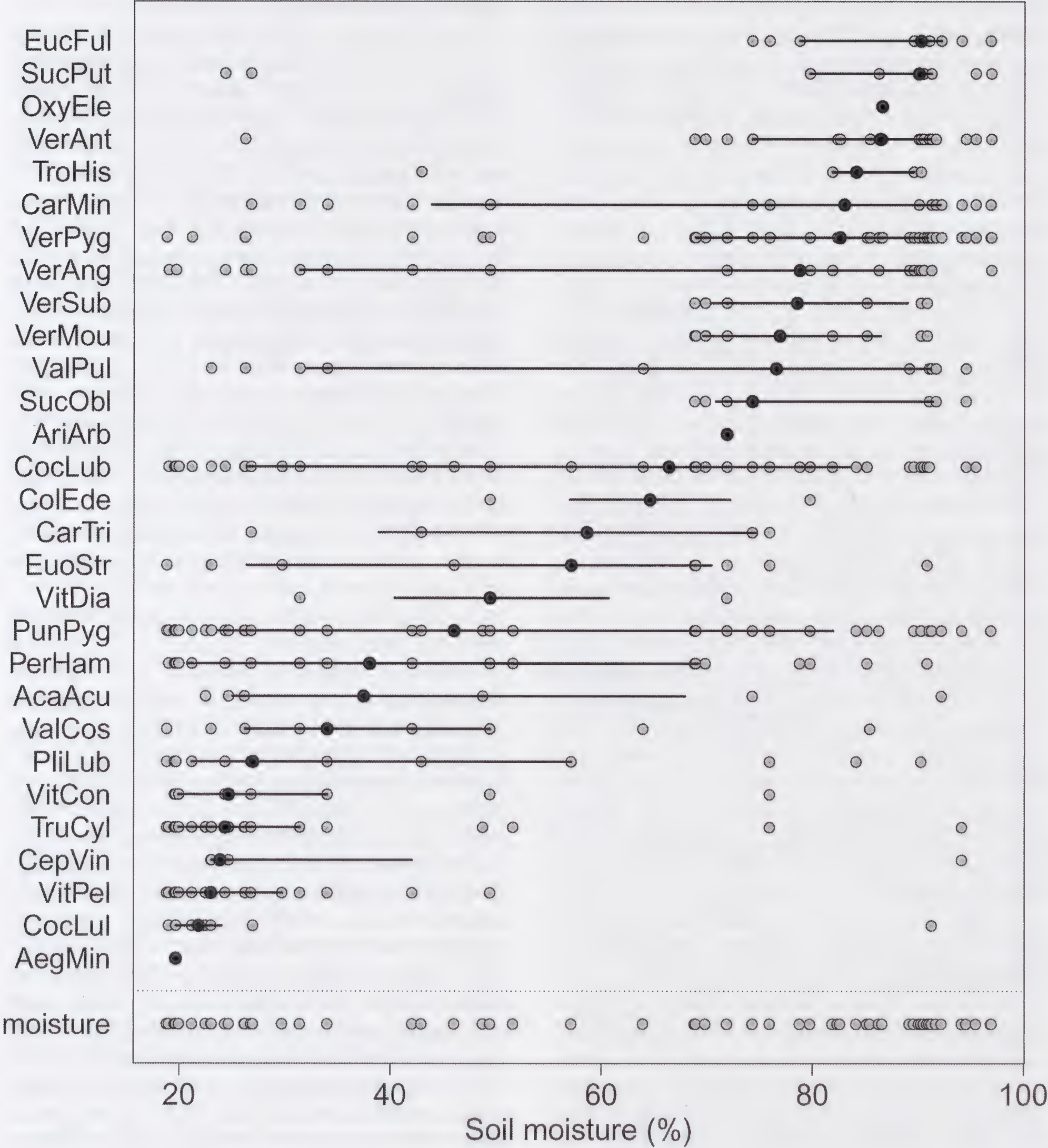


FIG. 4. Species occurrences (presence/absence data) along soil moisture gradient. Median (black point), 25–75% quantile (black line) and measured values of moisture where the species occurred (gray point) are displayed. Species are ordered by median values; for full species names, see Table 1. Distribution of measured moisture values are shown at the bottom.

classified sites into few moisture classes and found significant increase of species richness and abundances with increasing site basicity only in intermediate-moist and wettest forest sites. Also dry grassland habitats did not show any significant response of both species richness and total abundances to soil pH.(Martin & Sommer, 2004a). Getz & Uetz (1994) estimated

site moisture using a degree of slope and aspect, and they found a unimodal response of species richness to moisture, although when sites with low diversity of leaf litter were omitted, a positive linear response was obtained. It seems that there is no general pattern, especially if looking at ecologically highly different habitats harbouring evolutionary distinct

communities. In such a broader-scale overview, we can document species rich assemblages at both rather xeric (e.g., limestone rocks) and humid (e.g., calcareous fens) habitats (Nekola, 2002; Horsák & Cernohorsky, 2008). Interestingly, even for very straightforward response of snail species richness to the gradient of calcium content, important idiosyncrasies have been recently reported from North American faunas by Nekola (2010). He documented surprisingly species rich assemblages in some acidic habitats harbouring many strictly acidophile land snail species. Nevertheless, at fine scales a tight response of snail species richness to moisture cannot be rejected. Therefore, on the basis of previously published results we expected two possible responses of species richness to soil moisture in our study system – a decreasing linear response toward drier plots or a unimodal hump-shaped pattern with a peak at intermediate values. In contrast, we found only non-significant (after a Bonferroni correction) negative correlation, which can have several possible reasons. First is a lack of permanently dry sites, because the studied grasslands are usually classified as semi-dry. They occur on flysh bedrock, which is rich in clay particles capable of absorbing water and thus keeping moisture in dry periods. This results in a common co-occurrence of both moisture-demanding and dry-grassland plant species (Škodová et al., 2008). We have observed similar patterns for snails as well. For example, several live individuals of wetland snail *Vertigo angustior* was found in a semi-dry grassland approximately 20 m far from the edge of the fen community, though in the study region it is exclusively limited to alkaline wetlands. It illustrates the second reason: dry grasslands are not spatially isolated from fen communities, and these may act as a part of the source-sink dynamics (Shmida & Ellner, 1984). Third, the observed negative correlation between moisture and species richness may be caused by covariation with biomass production. We observed higher biomass production of vascular plants in dry-grassland plots, presumably due to improved nutrient availability as compared to phosphorus-limited fens (Rozbrojová & Hájek, 2008). Low-productive vegetation of calcareous fens did not provide suitable shelters for land snail survival during unfavourable periods (Schamp et al., 2010) and is probably also less palatable because of prevalence of sedges over herbs and grasses (Schamp et al., 2011).

Species Composition

In contrast to species richness and abundances, we observed remarkable change of species composition over the gradient. Particular species differed in their optima along the gradient, with five species (after a Bonferroni correction, Table 1) preferring wetter plots, other three species being more abundant in drier plots. Only two species experienced a unimodal response, though their optima were shifted towards wetter (*Vertigo moulinsiana*) or drier (*Vallonia costata*) plots. This clearly suggests a species turnover from drier to wetter plots rather than species accumulation towards wetter plots. Similar result was found in the Danube floodplain forests, from where Čejka et al. (2008) reported a great effect of moisture on snail species composition, but not on species richness and total abundances. Chiba (2007) reported the coincidence between moisture and snail species richness as well, but contrary to our study, he also found a hump-shaped relationship between moisture and species richness. However, he did not measure or estimate moisture, but used the first DCA axis as its proxy. We can demonstrate how this approach may be misleading. In concordance, our data also clearly show a hump-shaped relationship between the species richness and the plot scores on the first DCA axis (Fig. 3), which was tightly associated with soil moisture (Table 5). Despite that, there was no significant relationship between moisture and any measure of species richness in our study. This pattern thus seems to be driven by a confounding effect of a fine-scale species turnover associated with the well-known concept of ecotone (or rather ecocline in our study system), where species common to the adjacent assemblages are allowed to coexist (see van der Maarel, 1990). Such samples are inherently placed in the middle of the ordination axis, as their species composition results from meeting faunas of different ecologies, although they may not necessarily have intermediate values of soil moisture.

Species Responses

Using species response modelling, we obtained more or less different autecological conclusions for some species than was previously published from different habitats (Martin & Sommer, 2004b; Čejka & Hamerlík, 2009) and even from the same habitat sampled in a wider area and higher number of sites (Horsák

et al., 2007). However, it is important to stress that these results are hardly comparable. In the three above-mentioned studies, only one sample per study site was sampled, and ecological variation among the sites was much higher. The results can be further biased by confounding factors and biotic interactions. We therefore assume that our within-site design could at least partially control for some of these drawbacks of the traditional sampling scheme. Despite these circumstances, there were several species that expressed consistent response across different studies. *Vallonia costata*, for example, was always more common in drier plots. In addition, our autecological results are identical with those of Martin & Sommer (2004a), who sampled similar habitats using similar sampling scheme, that is, sampling in separate sites, but in a distinct region, which was homogeneous with respect to climate and environment characteristics.

Conclusions

In this study, we used the within-site sampling design to control for confounding effects other than soil moisture, which was measured directly. Even if moisture correlated with soil calcium in our sites, the amount of calcium was very high in all plots, making the effects of its variation for land-snail composition negligible (Horsák, 2006). Using this methodology, we observed clear compositional changes along the moisture gradient, suggesting differences in species preference to soil moisture conditions even at a fine, within-site scale. Species richness did not change, or changed slightly negatively, along the gradient. Although some of these results can be explained either by missing extremely dry plots or by specific features of our study system, it suggests that the effect of soil moisture on land snail communities is rather variable across ecologically different habitats and geographically and evolutionary distinct faunas.

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DIFFERENCES IN DENSITY, SHELL ALLOMETRY AND GROWTH BETWEEN TWO POPULATIONS OF *LIMNOPERNA FORTUNEI* (MYTILIDAE) FROM THE RÍO DE LA PLATA BASIN, ARGENTINA

Nicolás Bonel^{1*, 3}, Lía C. Solari² & Julio Lorda⁴

ABSTRACT

The invasive freshwater mussel, the mytilid *Limnoperna fortunei* (Dunker, 1857), has a great capacity for colonizing a wide range of aquatic environments because of its dispersal ability, high fecundity and wide range of physiological tolerances. Most of the biological and ecological studies of *L. fortunei*, having been restricted to specific locations, lack comparative analyses among different habitats. In this investigation, we examined the differences in larval density, density in settlement plates, shell allometry, and growth between two populations from the Río de la Plata basin, Argentina. One of the populations inhabited a heavily polluted area, whereas the other a moderately polluted area. We predicted that the density and growth of the golden mussel would be lower in the heavily polluted environment, expecting therefore to find variations in shell allometry as a consequence of differences in density and environmental conditions between the sites investigated. We accordingly found that the larval density, the density of settled individuals, and the growth were lower in the more polluted environment. We also observed allometric differences because the individuals from the moderately polluted area with higher population densities were more elongated (i.e., with a higher shell length-to-width ratio). The golden mussel tolerates a wide range of environmental conditions and can survive in many polluted water bodies where other invasive species cannot. The findings presented here support the idea that *L. fortunei* can inhabit heavily polluted environments, but at the expense of a significant decrease in its biologic potential.

Key words: invasive species, freshwater mussel, population dynamics, growth rate, water pollution.

INTRODUCTION

Population size – one of the key parameters determining the economic and ecologic impacts of an invasive species – may vary over space and time (Strayer & Malcom, 2006). Information concerning the influence of environmental conditions on growth, survival and fecundity of aquatic species is necessary for making predictions about the potential for populations to expand and exploit new habitats (Garton & Johnson, 2000).

The freshwater bivalve *Limnoperna fortunei* (Dunker, 1857) is native to Southeast Asia and has been introduced into other regions of Asia

and South America (Boltovskoy et al., 2009a). Known as the golden mussel, *L. fortunei* has become a pest species of global concern over the last few decades because of its major economic impacts on the industrial and power plants that use raw river or lake water for their processes (Ricciardi, 1998; Boltovskoy et al., 2009b), and as a result of its ecological impacts upon the native communities of mollusks and crustaceans (Lopes et al., 2009). In Argentina, *L. fortunei* was first detected in 1991 in the brackish waters of the Río de la Plata estuary (Pastorino et al., 1993). Shortly thereafter, this mytilid expanded its range of settlement throughout more than 3,000 km of the Río

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de la Plata-Paraná-Paraguay river system (reviewed in Boltovskoy et al., 2009a). The geographical dispersion of *L. fortunei* is largely facilitated by its planktonic larvae (Cataldo & Boltovskoy, 2000). This freshwater mytilid shares several biologic and ecologic features with the European and North American invasive pest mollusk, the zebra mussel, *Dreissena polymorpha* (Pallas) (Karatayev et al., 2007). Like *D. polymorpha*, the golden mussel possesses many of the characteristics attributed to successful invaders, that is, a short generation time, phenotypic plasticity, gregariousness, abundance in its native habitat, wide environmental tolerance and commensal association with human activity, all of which characteristics make its potential spread and establishment in North American waters very likely (Ricciardi, 1998; Oliveira, 2010a).

The population dynamics of the bivalves depends on the successful settlement and recruitment of dispersed larvae (Martel et al., 1994). During the reproductive period of *L. fortunei* (September through April), the density of the settled individuals can exceed 200,000 mussels/m² because of the high abundance of recently settled juveniles, whereas the density of the subadult and adult individuals usually ranges between 5,000 and 10,000 mussels/m² (Sardiña et al., 2009). High population density promotes changes in shell allometry as the result of a regulation in food intake, some form of physical interference, or an interaction between the two (Alunno-Bruscia et al., 2001). In spite of the high densities and extensive conglomerations that are characteristic of *L. fortunei*, no studies have as yet looked at the shell allometry and analyzed its relationship to population density. Only Morton (1977) in Hong Kong has estimated the ratios of shell width to height to length in *L. fortunei*, but he did not perform a comparative analysis of shell allometry among different populations.

Variations in growth often are habitat-specific and directly related to the local environmental conditions (Alfaro et al., 2008). An analysis of growth rates under natural conditions is a necessary first step in understanding the growth dynamics and energy balance of a given bivalve population (Dorgelo, 1992). Previous studies have estimated the growth of *L. fortunei* individuals in the Neotropical Region and in Asia through different methods; those investigations, however, were limited to only one location per study (Boltovskoy & Cataldo, 1999; Maroñas et al., 2003; dos Santos et al., 2008; Belz et al., 2010; Darrigran et al., 2011;

Nakano et al., 2011). Magara et al. (2001) analyzed the growth of *L. fortunei* in two Japanese water systems, but they performed no comparative analyses of the growth between the two environments. Oliveira et al. (2011) compared the growth of two populations in Brazil but found no differences.

Most of the biological and ecological studies of *L. fortunei* were restricted to specific locations and therefore lacked comparative analyses (Boltovskoy et al., 2009a). Moreover, virtually no information is available on the ecological preferences of *L. fortunei*, and our knowledge of its limnologic and biologic traits within the enormous and very complex South American river system is still limited (Boltovskoy et al., 2006). In this study, we investigated the differences in larval density, density on settlement plates, shell allometry, and growth between two populations of *L. fortunei* from two river systems from the Río de la Plata basin. Since this species can inhabit ecosystems with widely differing environmental characteristics, we predicted that the density and growth of *L. fortunei* would be lower in heavily polluted environments. Furthermore, as a consequence of such differences in population densities and environmental conditions, we expected that individuals from high densities and moderately polluted habitats would have elongated shells.

Information on the spatial variability in the ecological aspects related to the life-history of *L. fortunei* in response to different environmental conditions would contribute to the existing data garnered from specific locations. A more comprehensive understanding of the biologic parameters of this invasive freshwater mussel in the face of potentially stressful conditions would help us to predict the effect scenarios such as climate change and eutrophication might have on this mytilid's life-history traits and subsequently its impact on native invaded ecosystems.

METHODS

Study Sites

To perform this study, we selected two water bodies from the Río de la Plata basin, Argentina. The first site was in the Coronda River, a secondary course of the Paraná River (31°41'26.88"S, 60°44'34.08"W). The Coronda is close to two urban centers with approximately 600,000 inhabitants who release various urban and industrial discharges as well as rural run-

offs constituting major sources of organic matter (Devercelli & Peruchet, 2008). The second site was the Santiago River (34°51'5.76"S, 57°53'29.76"W) located 350 km southeast of the first sampling point. This river is an 8 km-long tributary of the Río de la Plata estuary and is close to two extensive urban centers with more than 11 million inhabitants that, in turn, are located along the first 80 km of the south shore of the Río de la Plata estuary (Colombo et al., 1989).

Environmental Parameters

We measured water temperature (T), pH, dissolved oxygen (DO), and conductivity with a digital multimeter (Sper Scientific 850081). Water clarity was measured with a Secchi-disk. The calcium concentration was determined in the laboratory by a volumetric method involving a Na₂EDTA solution and a murexide indicator (APHA, 1998). For photosynthetic-pigment analyses, we sampled 1–2 L of water, transferred the water samples to dark plastic bottles, and transported them to the laboratory in a cooler with ice to avoid chlorophyll photo-oxidation. The techniques, procedures, and assessments of chlorophyll concentration per cubic meter were carried out according to APHA (1998). We took weekly and monthly measurements of environmental parameters in the Santiago and Coronda rivers, respectively. To stabilize the variances and improve normality, we used the natural logarithm to transform all environmental data. To compare physicochemical and biologic parameters from both sites, we used two-tail pairwise *t*-tests. A discriminant analysis was applied to the quantitative data. Environmental variables were used in the multivariate analysis as explanatory parameters with respect to differences between two levels of an *a-priori* defined categorical variable (i.e., the study sites).

Larval Density

At both study sites, we filtered 400–500 L of water through a Wisconsin net (47 µm mesh size) using a water pump to obtain a total of 15 samples in the Coronda River from April 2007 through February 2009 and 14 samples in the Santiago River from March 2007 through March 2009. The particulate material retained by the net in a 0.5 L was placed in a receptacle and preserved in 40% (v/v) aqueous ethanol. We analyzed three to four 1 mL aliquots (sub-samples) from each sample. Each subsample

was placed in a 1 mL Sedgwick-Rafter chamber and observed with a stereoscopic microscope. To compare larval densities, we calculated the total density per cubic meter and then performed two-tail pairwise *t*-tests. The larval densities were squared-root transformed to meet the assumptions of normality and homoscedasticity. To evaluate associations between environmental variables and larval densities, we used Pearson's correlation coefficient.

Density on Settlement Plates

In order to calculate field densities, we deployed 36 settlement plates per river. We grouped the settlement plates – 10 x 10 cm square frames with 1 mm plastic mesh screens – in 12 blocks with three plates on each block and placed the settlement plates 1 m below the surface. At each study site, we regarded the sampling date prior to the first evidence of settlement as the initial date in the study. We collected data in the Santiago River from December 2007 through April 2009. In the Coronda River, we had to divide the study into two stages because six groups of collectors were lost. We collected data from October 2007 through June 2008 and then from September 2008 through February 2009. Once we collected the settlement plates, we transported the samples immediately to the laboratory in plastic bags. The individuals were gently detached from the collectors, washed over a sieve (1 mm pore size), and preserved in 70% (v/v) aqueous ethanol (Nakano et al., 2011).

To evaluate the relationship between density and larval settlement with the environmental parameters, we used Pearson's correlation coefficient. To meet normality and homoscedastic conditions, we transformed the density values to square roots, the percentage data to arcsine square roots, and calculated the natural logarithms of the environmental parameters. To compare densities from both study sites, we used a two-tail pairwise *t*-test.

Shell Allometry

We measured the length (L: mm, maximum anteroposterior axis) of all individuals collected from the settlement plates. To analyze and compare shell allometry between individuals from each site, we used only mussels longer than three millimeters. For these mussels we also measured the height (H: mm, maximum dorsoventral axis) and width (W: mm, maximum lateral axis; Alunno-Bruscia et al., 2001). We

used a Mitutoyo digital caliper (precision, 0.01 mm) for all measurements.

For each block, sampling date, and study site, we calculated the means of the morphometric variables and examined the allometric relationships of height and width as a function of length. We tested each pair of size variables with the allometric equation $y = ax^b$, where a and b are the coefficients of the equation. The correspondence of allometric curves fitted to mussels from the two rivers was tested through the use of the sum of the squares from the F test. A nonsignificant F -statistics indicated that a single allometric curve was sufficient to describe the populations being compared (Blanchard & Feder, 2000). We also analyzed the effect of spatial variation on morphometric variables using two independent ANCOVAs with height and width as dependent variables, length as a covariable, and sites as factors. Shell dimensions were transformed by the natural logarithm to homogenize variances.

To investigate changes in shell morphology, we calculated the morphometric ratios between the linear shell dimensions, length-to-width (L/W), length-to-height (L/H), and height-to-width (H/W) (Alunno-Bruscia et al., 2001). We transformed the ratios to arc-sine square roots to meet normality and homoscedastic conditions, and used two-tail t -tests to make comparisons between the groups. To analyze the relationship between population density and changes in morphometric ratios, we used Pearson's correlation coefficient.

Growth

To assess growth, we used length-frequency distributions with reference to the average shell length. We calculated the range of each length-frequency distribution as the difference between the maximum and minimum value of the frequency data. The equation to calculate the number of the classes is:

$$k = 1 + \log_2 n$$

where k is the number of the class interval and n is the sample size. The equation for class interval of width (h) is:

$$h = (\text{range} + \mathcal{E}) / k$$

where $\mathcal{E} = 10\%$ difference from the range of values (i.e., minima and maxima values) (Sturges, 1926).

To identify cohorts by means of the length-frequency distributions, we applied the Bhat-tacharya (1967) method available in FISAT II software (Version 1.1.2, FAO-ICLARM Fish Assessment Tools; Gayanilo et al., 1996). To

confirm each modal progression, we used the NORMSEP method also available in the FISAT II software (Pauly & Caddy, 1985). Depending on shell length, we defined two categories of individuals: (1) juveniles (length < 5 mm, the minimum size for sexual differentiation; Dar-rigran et al., 1999); (2) subadults and adults (length > 5 mm).

To describe and compare the growth between both sites, we fitted linear and nonlinear regression models (quadratic and cubic) (Smit et al., 1992). Each fit was tested according to the sum of the squares from the F test. The null hypothesis was that the simpler model (the one with fewer parameters) was correct. If $P < 0.05$, the null hypothesis is rejected (i.e., the simpler model is discarded), and the conclusion becomes that the more complex model fits the data group significantly better. Likewise, we used this test to compare the parameters from each growth curve of the two populations from the two rivers. A nonsignificant F -statistics indicated that a single allometric curve was sufficient to describe the populations being compared (Blanchard & Feder, 2000). All statistical analyses were performed by means of the SPSS 17.0 statistical package, JMP statistical software (v9.0 SAS Institute), and GraphPad Prism 5 (www.graphpad.com).

RESULTS

Environmental Variables

Table 1 summarizes the descriptive statistics of the environmental variables measured at both study sites. Pairwise comparisons showed significant differences in water temperature between the two study sites ($t_{(17)} = -3.38$, $P = 0.0036$). On the average, the Coronda River was warmer than the Santiago by 1.2°C. The dissolved oxygen was significantly different between the Coronda and Santiago rivers ($t_{(16)} = -7.99$, $P < 0.0001$). The Coronda River exhibited, on the average, a 3.4 mg/L higher concentration of dissolved oxygen than the Santiago. Conversely, the conductivity, at an increment of 0.209 mS/cm, was significantly higher in the Santiago River ($t_{(17)} = 6.49$, $P < 0.0001$). Both sites were characterized by slightly alkaline water, but the pairwise comparisons revealed that the pH was nevertheless significantly different ($t_{(17)} = -3.04$, $P = 0.0074$) between the two rivers, with the Coronda River being an average of 0.14 units more alkaline than the Santiago. Although at both sites the

TABLE 1. Mean value ± standard deviation (minimum-maximum; n, number of samples) of the environmental variables measured at both study sites and the pairwise *t*-test results. T, water temperature (°C); DO, dissolved oxygen (mg/L); Cond, conductivity (mS/cm); Secchi, transparency (m); Ca, calcium concentration (mg/L); Chl a, chlorophyll a (mg/m³); Chl b, chlorophyll b (mg/m³); Chl c, chlorophyll c (mg/m³).

	Coronda River	Santiago River	Paired <i>t</i> -test
T	20.7 ± 5.8 (10.1–29.5) n = 18	19.5 ± 5.7 (9.5–27.6) n = 23	$t_{(17)} = -3.38, P = 0.0036$
pH	7.55 ± 0.22 (7.16–8.0) n = 18	7.41 ± 0.12 (7.17–7.54) n = 23	$t_{(17)} = -3.04, P = 0.0074$
DO	8.6 ± 2.4 (6.0–14.8) n = 18	5.2 ± 1.7 (2.8–10.3) n = 22	$t_{(16)} = -7.99, P < 0.0001$
Cond	0.223 ± 0.144 (0.081–0.655) n = 18	0.432 ± 0.050 (0.327–0.509) n = 23	$t_{(17)} = 6.49, P < 0.0001$
Secchi	0.3 ± 0.1 (0.1–0.5) n = 15	0.5 ± 0.1 (0.3–0.8) n = 21	$t_{(14)} = 4.13, P = 0.001$
Ca	48 ± 46 (6–180) n = 13	53 ± 27 (13–88) n = 17	$t_{(11)} = 1.32, P = 0.2111$
Chl a	6.9 ± 5.6 (2.6–22.9) n = 13	17.2 ± 12.7 (0.9–45) n = 20	$t_{(12)} = 3.01, P = 0.0109$
Chl b	3.0 ± 5.6 (0–21.1) n = 13	10.8 ± 18.3 (0–82.1) n = 20	$t_{(12)} = 3.17, P = 0.008$
Chl c	2.8 ± 3.5 (0–12.9) n = 13	4.2 ± 4.6 (0.1–18.4) n = 20	$t_{(12)} = 0.22, P = 0.8316$

water clarity was low, the Coronda River being 0.2 m less transparent than the Santiago, exhibited a significant difference in turbidity ($t_{(14)} = 4.13, P = 0.001$). The calcium concentration was comparable between the two sites ($t_{(11)} = 1.32, P = 0.2111$). The Santiago River had an average of 10.3 and 7.8 mg/m³ higher concentrations of chlorophyll a ($t_{(12)} = 3.01, P = 0.0109$) and b, respectively, than the Coronda ($t_{(12)} = 3.17, P = 0.008$), while the chlorophyll c was equivalent ($t_{(12)} = 0.22, P = 0.8316$).

Discriminant analysis gave a 95.1% correct classification. The canonic correlation coefficient from discriminant function 1 was 0.962 – that is, the proportion of variance resulting from the difference between groups (the coef-

ficient² x 100) was 92.5%. The score values for the Santiago River (centroid at -2.837 on the discriminant eigenvector) ranged from -6.10 to -0.232, but for the Coronda River (centroid at 4.019) from -2.011 to 5.541. Based on a 0.30 cut-off discriminant loading criterion, from all variables entered into discriminant analysis, the structure matrix revealed only two physico-chemical variables that significantly loaded on the discriminant function: conductivity (-0.557) and dissolved oxygen (0.319; Fig. 1).

Larval Density

A pairwise comparison revealed that the larval density (LD) was significantly higher in

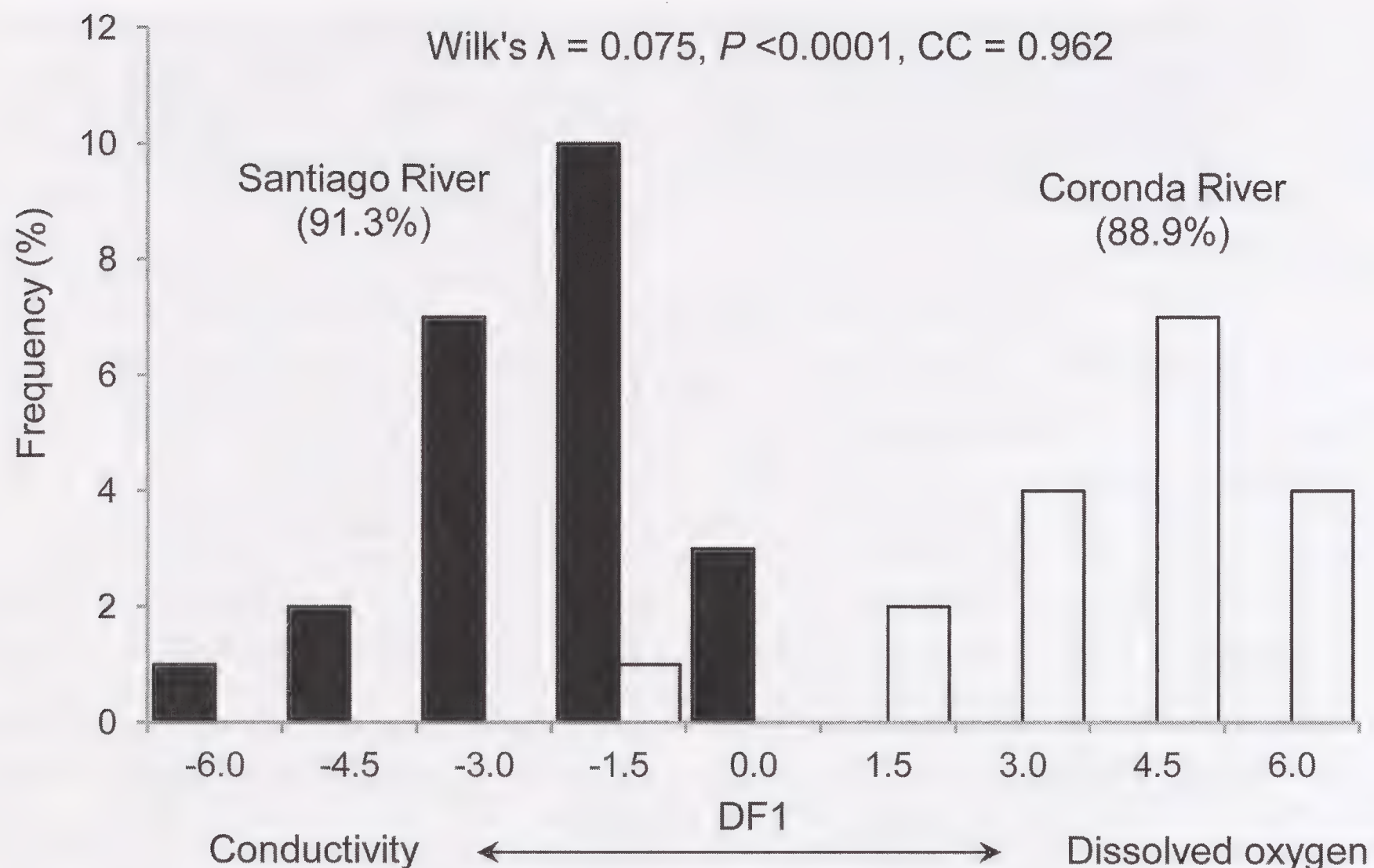


FIG. 1. Frequency histogram of discriminant function 1 (DF1) corresponding to the discriminant analysis through the use of biotic and abiotic parameters from the Coronda and Santiago rivers. The correct classification percentages, Wilks's λ , and canonical correlation (CC) are indicated.

the Coronda River than in the Santiago ($t_{(6)} = -3.402$, $P = 0.0145$; Fig. 2a). Notwithstanding, the difference estimated could have been heavily influenced by a single sample in December 2007 in the Coronda River (Fig. 2a). We therefore performed a pairwise comparison excluding the paired data, but the difference in the larval densities between the two rivers still remained significant ($t_{(5)} = -3.985$, $P = 0.0105$). In the Coronda River, the density attained the highest values in December 2007 ($20,240 \pm 7,825$ ind/m³). During 2008, we observed two main density peaks, in April ($5,320 \pm 1,773$ ind/m³) and in December ($4,800 \pm 462$ ind/m³), but in each instance the density values were four-fold lower than in December 2007. We found the lowest densities, < 500 ind/m³, when temperature was below 15°C (Fig. 2a). We found a positive correlation between larval density and temperature ($LD = -230.77 + 92.70 T$, $r = 0.784$, $P < 0.0001$) and a negative correlation with dissolved oxygen ($LD = 271.46 - 106.34 DO$, $r = -0.7576$, $P < 0.001$). The remaining environmental variables showed no significant correlation with density.

In the Santiago River, we observed two main larval density peaks during 2007, one in May ($3,000 \pm 265$ ind/m³) and another in December ($2,600 \pm 902$ ind/m³). A single peak occurred in

April 2008 ($3,420 \pm 570$ ind/m³), while in 2009 the peak was recorded in March ($2,625 \pm 573$ ind/m³). The larval density in the water column was also lower than 500 ind/m³ when water temperatures were below 15–16°C (Fig. 2a). We found a positive correlation between larval density and temperature ($LD = -125.91 + 50.93 T$, $r = 0.589$, $P = 0.0265$). Contrary to what we observed in the Coronda River, we found no significant correlation between the dissolved oxygen levels and the larval density.

Density on Settlement Plates

A pairwise comparison revealed that the total density (TD) of settled mussels was significantly lower in the Santiago River than in the Coronda River ($t_{(6)} = -3.056$, $P = 0.0223$; Fig. 2c). In the Santiago River, the highest density occurred in April 2007 ($31,900 \pm 13,323$ ind/m²), when 90% of the individuals sampled were juveniles (Juv; Fig. 2b). The lowest density ($1,167 \pm 551$ ind/m²) was observed in December, with juveniles representing 46% of the total individuals. In 2009, the highest peak was recorded in March ($13,200 \pm 8,728$ ind/m²), with 60% being juveniles (Fig. 2b). We found both a positive correlation between the total density and the percentage of juveniles ($Juv =$

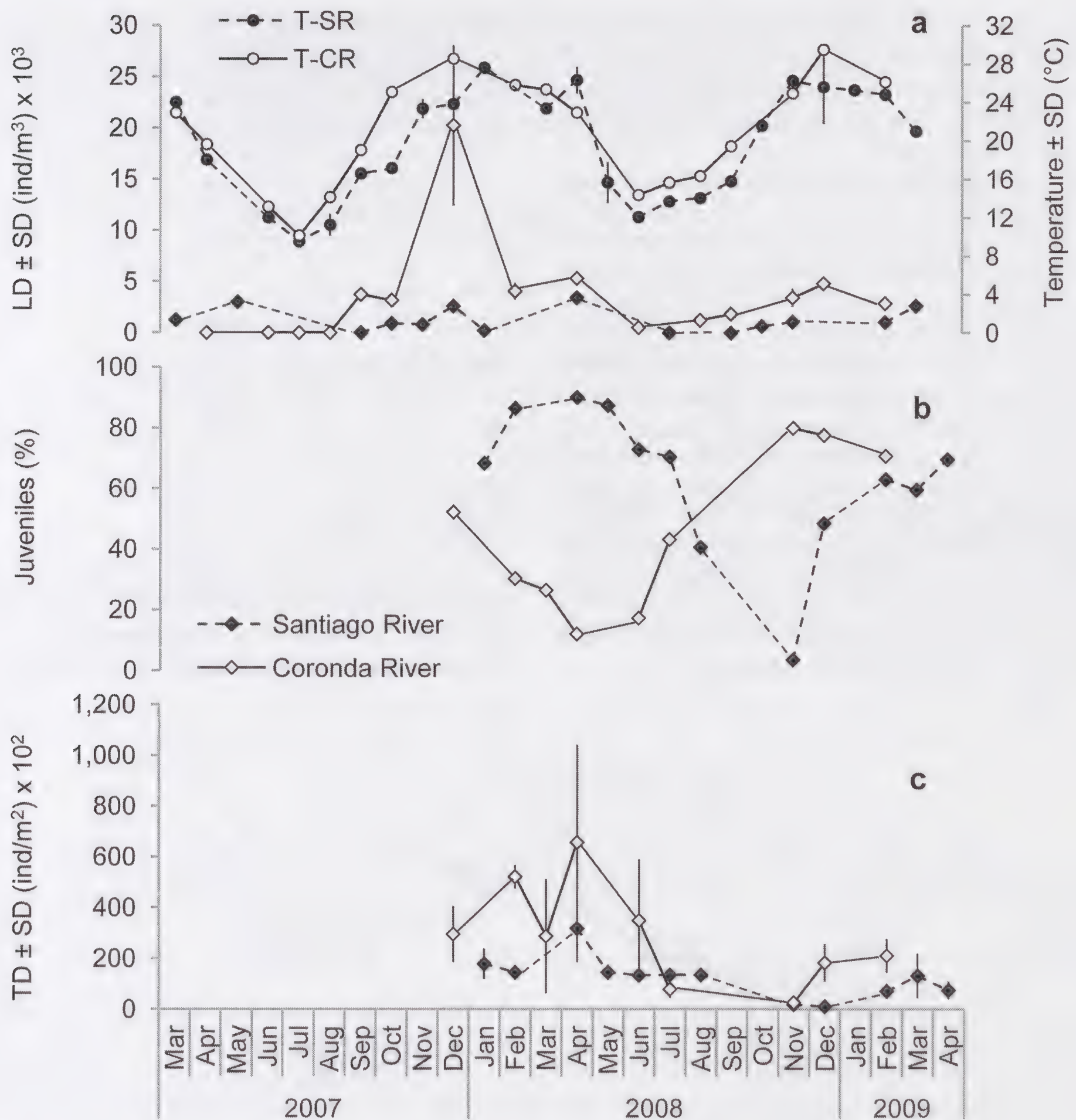


FIG. 2. Seasonal variation of *Limnoperna fortunei* and water temperature (T) $\pm$ standard deviation (SD) from the Coronda (CR) and Santiago (SR) rivers with respect to a: Larval density, LD $\pm$ SD; b: Percentage of juveniles (%); c: Total density of settled individuals, TD $\pm$ SD.

$1.909 + 0.0071 \text{ TD}$, $r = 0.6077$, $P = 0.0361$) and a negative correlation between the total density and the pH ($\text{TD} = 4,169.65 - 544.54 \text{ pH}$, $r = -0.6679$, $P = 0.0247$). The remaining biotic and abiotic variables showed no significant correlation with density.

In the Coronda River, the highest density was found in April 2008 ($65,700 \pm 38,466 \text{ ind/m}^2$) during the first stage of the study. Contrary to what we observed in the Santiago River, the juveniles represented only 12.1% of the total individuals. The lowest density ($7,933 \pm 3,347 \text{ ind/m}^2$) occurred at the beginning of winter (July), but at this time the juveniles had increased

to 43%. In the second stage, the first block contained $2,768 \pm 321 \text{ ind/m}^2$, and 80% were juveniles (Fig. 2b–c). The highest mean density occurred in February 2009 ($21,100 \pm 6,630 \text{ ind/m}^2$), at which time the juveniles represented 71% of the total. The average density in the first stage ($36,528 \pm 20,117 \text{ ind/m}^2$) was higher than in the second ($14,033 \pm 9,941 \text{ ind/m}^2$), but the mean percentage of juveniles was lower (30 ± 15 and $76 \pm 4\%$, respectively; Fig. 2b–c). We found a negative correlation between the total density and the percentage of juveniles ($\text{Juv} = 3.161 + 0.0041 \text{ TD}$, $r = -0.7612$, $P = 0.0172$). As the density increased, the fraction of juveniles

TABLE 2. Coefficients of allometry from the least-square potential regression ($y = ax^b$) and the linearized model ($\ln y = a + b \ln x$) based on values of height (H) and width (W) as functions of length (L) from the Coronda and Santiago rivers. R², coefficient of determination; ln, natural logarithm.

Model	Coronda River			Santiago River		
	a	b	R ²	a	b	R ²
$H = aL^b$	0.636	0.895	0.99	0.614	0.900	0.99
$W = aL^b$	0.377	0.996	0.99	0.312	1.110	0.99
$\ln H = a + b \ln L$	-0.466	0.902	0.99	-0.490	0.901	0.99
$\ln W = a + b \ln L$	-1.008	1.012	0.99	-1.140	1.097	0.98

decreased. Finally, we observed a negative correlation between the total density and the water clarity ($TD = 257.11 - 407.42 \text{ Secchi}$, $r = -0.7030$, $P = 0.0347$), but no significant correlation between the total density and any of the remaining biotic or abiotic variables.

Shell Allometry

We measured the shell dimensions of 1,850 individuals from the Santiago River and 5,281 from the Coronda. The measurements on the mussels from the Santiago River showed that

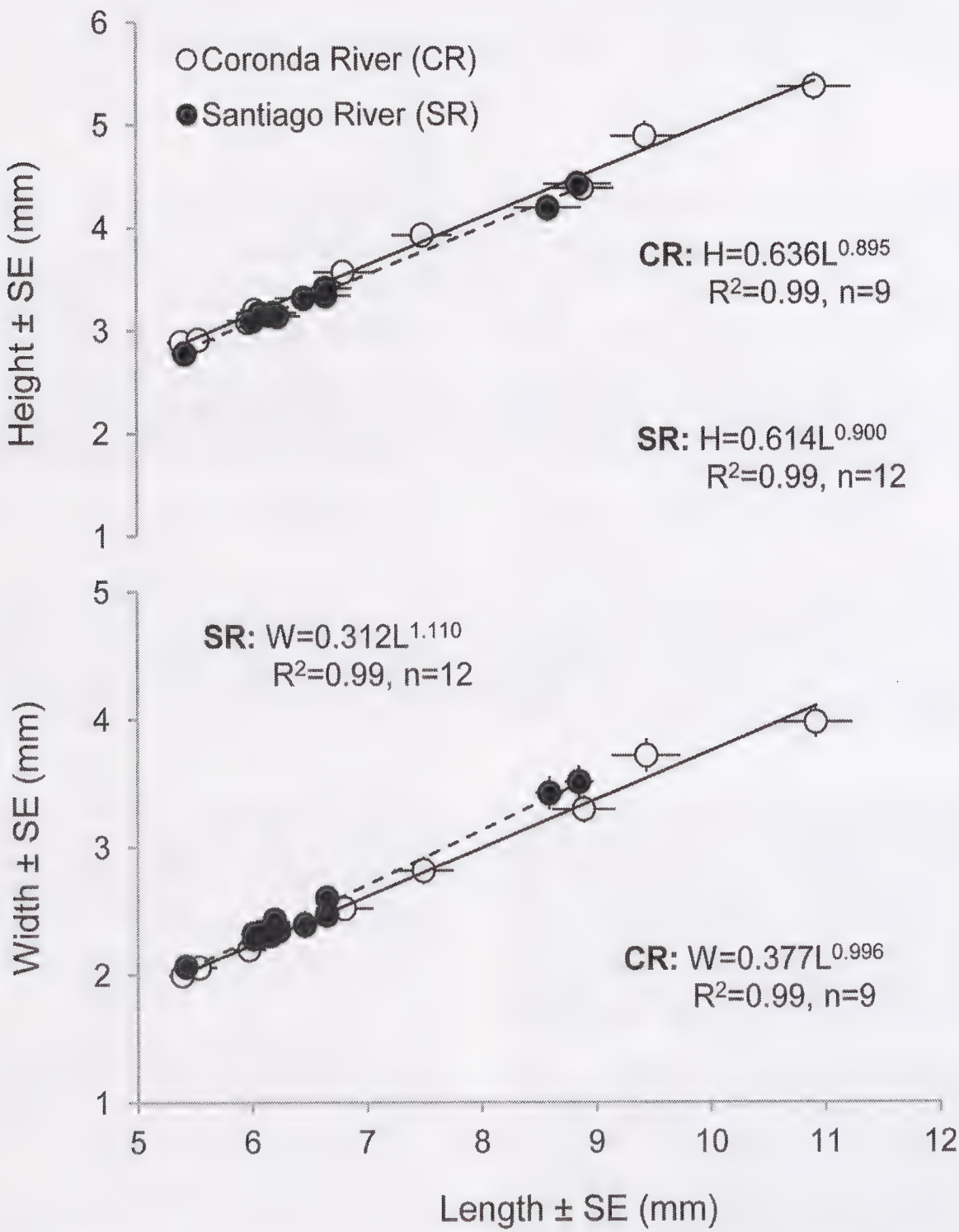


FIG. 3. Allometric regressions ($y = ax^b$) of mean values ± standard error (SE) of height (H) and width (W) as functions of length (L). Solid line: Coronda River; dashed line: Santiago River.

TABLE 3. Results of an analysis of covariance (ANCOVA) testing the spatial variability on ln-transformed shell dimensions (height: H and width: W) of *Limnoperna fortunei* as a function of sites (the Coronda and Santiago rivers) with the natural logarithm of shell-length as covariable.

Source	d.f.	MS	F	P
<i>lnH</i>				
Sites	1	0.0031	14.31	0.0014
lnL	1	0.6068	2778.78	< 0.0001
Error	18	0.0002		
<i>lnW</i>				
Sites	1	0.0044	8.47	0.0093
lnL	1	0.8045	1534.29	< 0.0001
Error	18	0.0005		

the shell length was 1.33 ± 0.05 and 2.61 ± 0.05 times greater than the height and the width, respectively; while the mussels from the Coronda River had shells of length 1.38 ± 0.04 and 2.68 ± 0.01 times greater than the height and the width, respectively. The shell length-to-height allometric relationships of the mussels from the Coronda and Santiago rivers were significantly different between the two sites ($F_{(2,17)} = 5.623$, $P = 0.0133$), but in both instances the models indicated a slight negative allometry (Table 2; Fig. 3). The shell length-to-width relationships for mussels from the Coronda River were consistent with isometry, while for individuals from the Santiago River the model showed a positive allometry (Table 2; Fig. 3). The allometric curves were significantly different between the two rivers ($F_{(2,17)} = 6.704$, $P = 0.0071$).

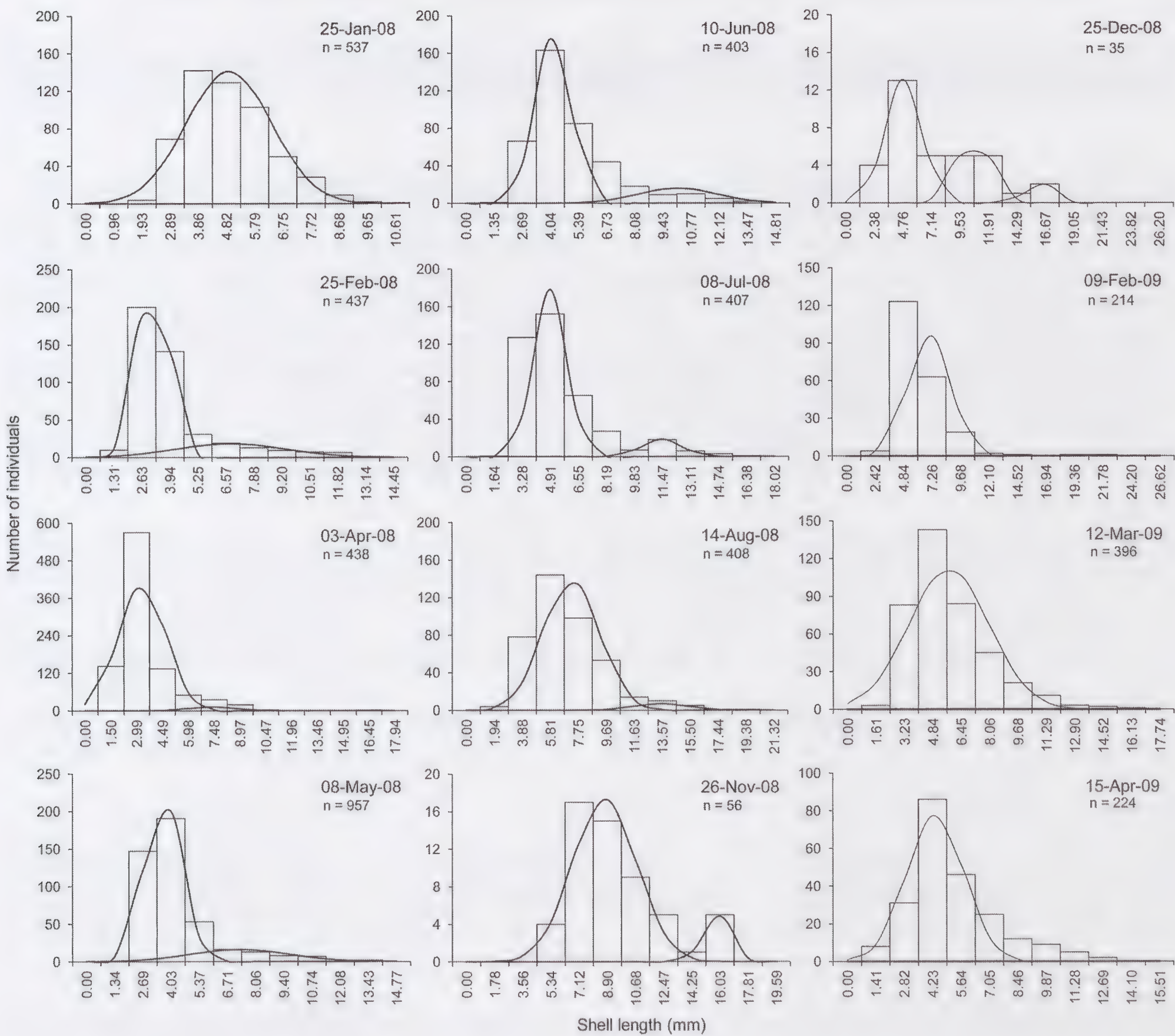


FIG. 4. Length-frequency distribution of *Limnoperna fortunei* from the Santiago River between January 2008 and April 2009; n: number of mussels measured.

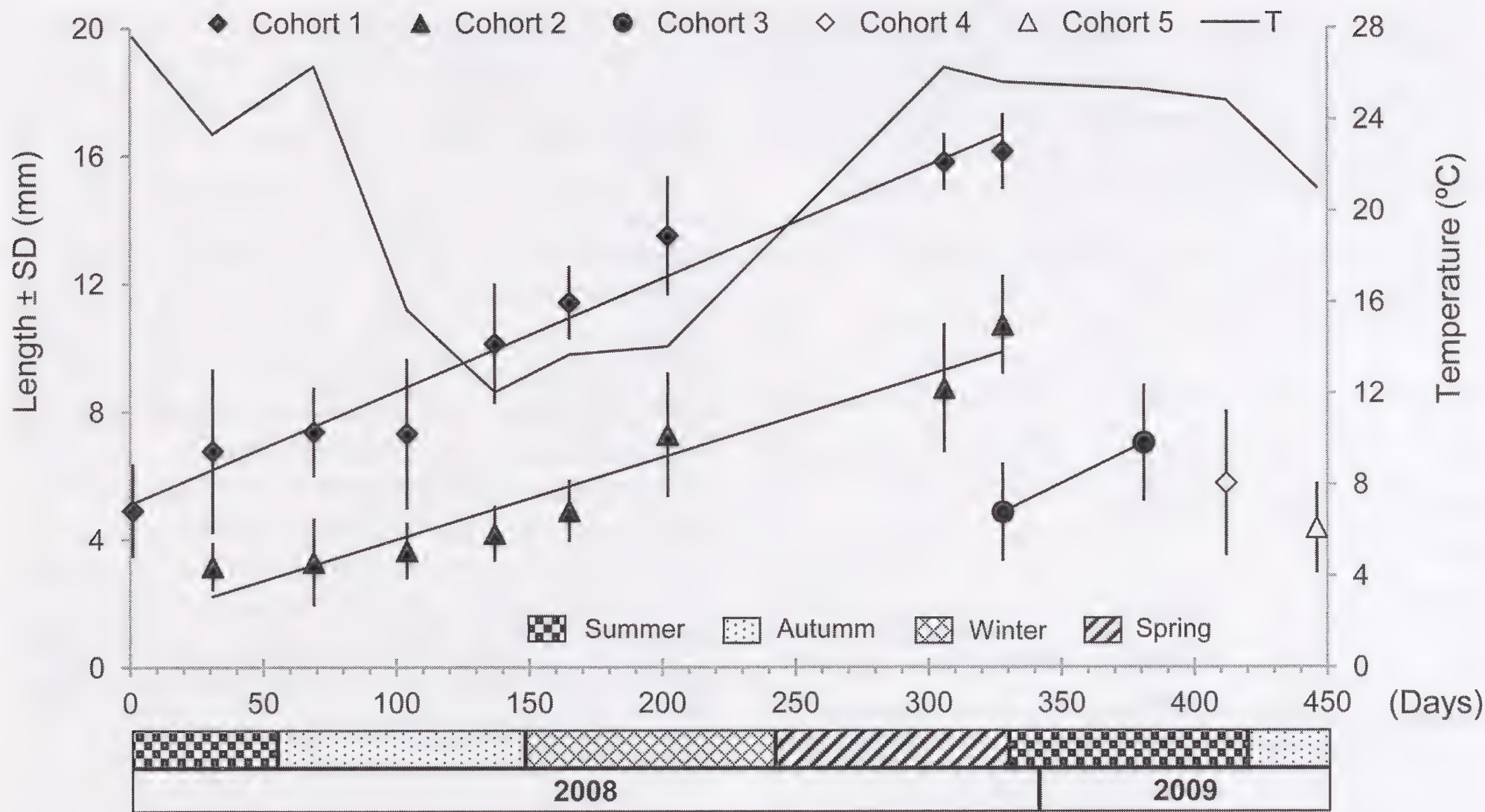


FIG. 5. Growth models fitted to the mean shell-lengths $\pm$ standard deviation (SD) of cohorts identified throughout the period of study in the Santiago River. T: water temperature.

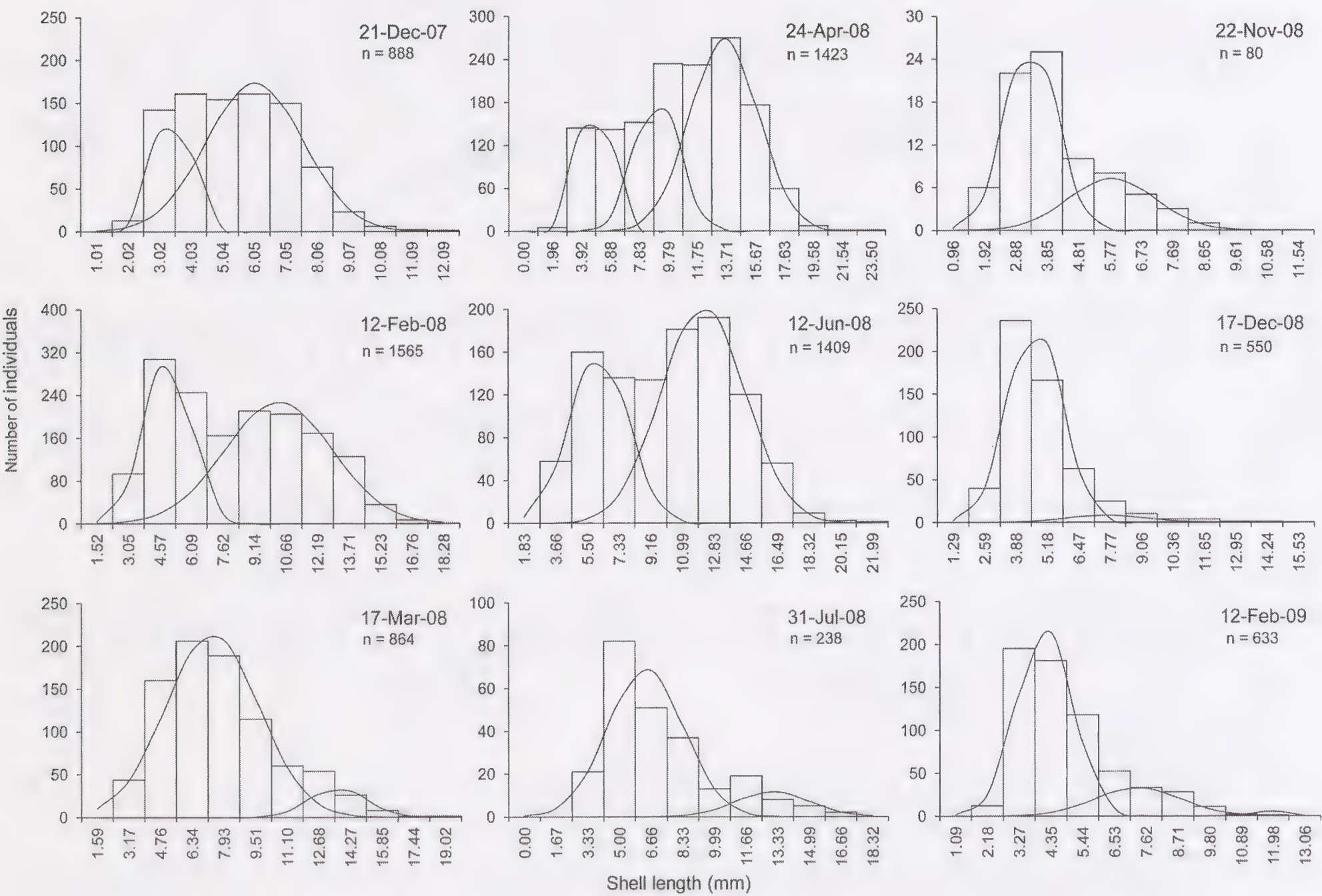


FIG. 6. Length-frequency distribution of *Limnoperna fortunei* from the Coronda River between December 2007 and February 2009; n: number of mussels measured.

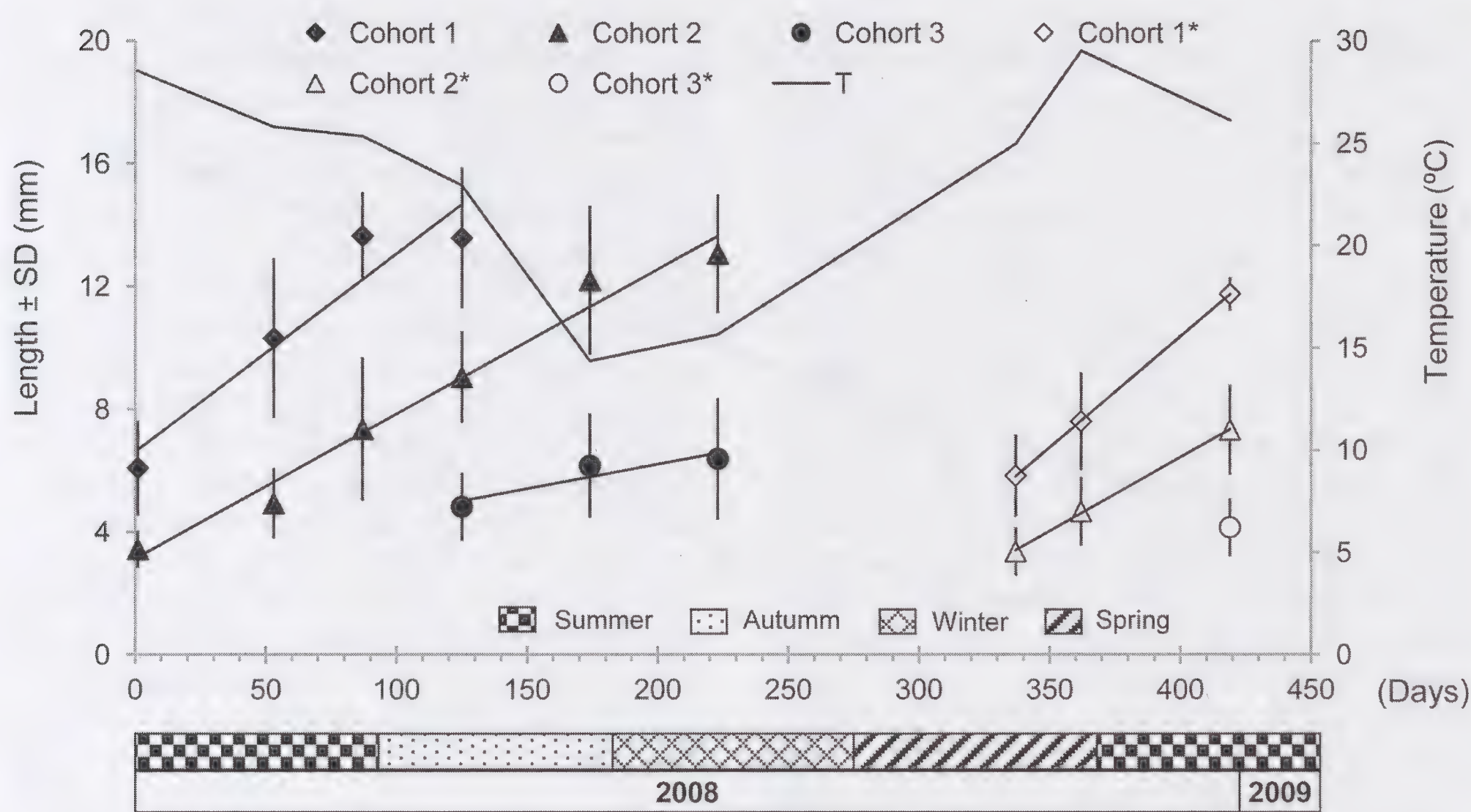


FIG. 7. Growth models fitted to the mean shell-lengths ± standard deviation (SD) of cohorts identified throughout the period of the first and second stage (*) of the study in the Coronda River. T: water temperature.

The sites had a significant effect on shell dimensions: mussels from the Coronda River exhibited a higher height and a lower width than individuals from the Santiago River (Table 3). In both instances no significant interaction occurred between the sites and the covariable shell length ($F_{(1,17)} = 0.0007$, $P = 0.979$, and $F_{(1,17)} = 2.3826$, $P = 0.141$; respectively). The t -test for the morphometric ratios indicated that the length-to-width was significantly higher –

that is, individuals were more elongated – in the Coronda River than in the Santiago ($t_{(19)} = -2.576$, $P = 0.0092$). We found no significant correlation between density and shell length-to-width values in the Coronda River, but the pooled density and shell length-to-width ratios from both study sites gave a positive correlation between the total density of settled individuals and the length-to-width ratio ($L/W = 1.0482 + 0.000135 \text{ TD}$, $r = 0.4512$, $P = 0.0401$).

TABLE 4. Results of the F -test performed to fit the best-growth model for each cohort. Parameters of the growth model ($y = a + bx$) fitted to each cohort of mussels from each site. R^2 : coefficient of determination, NA: data not available, (*): cohorts identified in the second stage.

Sites	Cohort	F test	Model	R^2
Santiago River (SR)	1	$F_{(1,6)} = 0.432$, $P = 0.536$	$y = 0.0353x + 5.10$	0.96
	2	$F_{(1,5)} = 2.651$, $P = 0.165$	$y = 0.0257x + 2.22$	0.93
	3	NA	$y = 1.573x + 1.41$	1.00
Coronda River (CR)	1	$F_{(1,1)} = 2.487$, $P = 0.360$	$y = 0.0645x + 6.61$	0.90
	2	$F_{(1,3)} = 0.102$, $P = 0.771$	$y = 0.0471x + 3.13$	0.98
	3	NA	$y = 0.0159x + 3.02$	0.85
	1*	NA	$y = 0.0723x - 18.55$	1.00
	2*	NA	$y = 0.0481x - 12.81$	1.00
SR vs. CR	1 vs. 1	$F_{(2,9)} = 20.33$, $P < 0.001$	Different curve for each data set	NA
	2 vs. 2	$F_{(2,10)} = 66.38$, $P < 0.001$	Different curve for each data set	NA

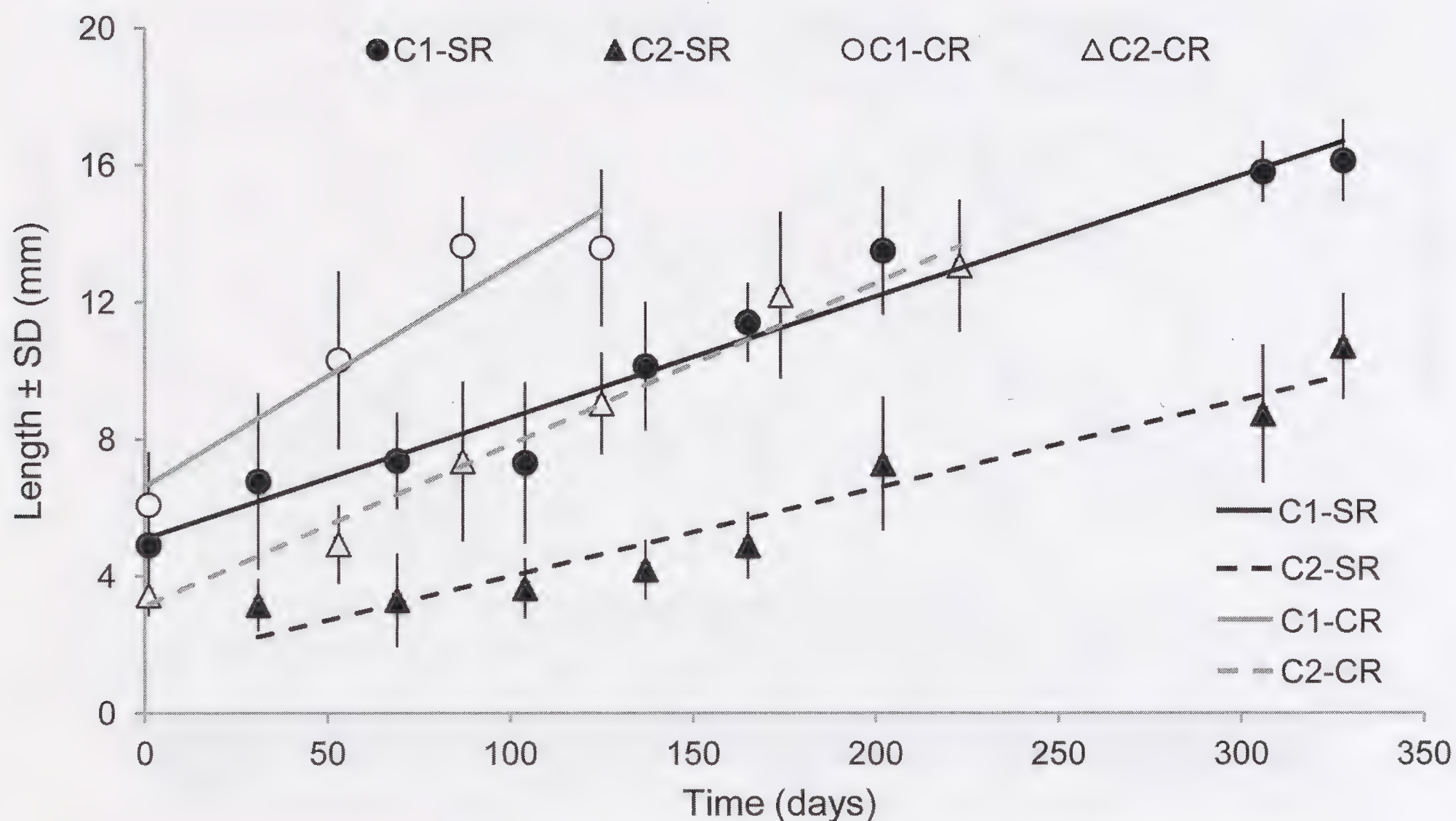


FIG. 8. Differences between the slopes of each growth model fitted to mean shell lengths $\pm$ standard deviation (SD) of cohorts 1 (C1) and 2 (C2) of *Limnoperna fortunei* from the Coronda and Santiago rivers.

Growth

We constructed length-frequency distributions based on the length values of 4,512 mussels from the Santiago River (Fig. 4). Polymodal decomposition identified five cohorts throughout the study period (Fig. 5). The F test did not reject the null hypothesis: cohorts 1 and 2 were better described by a linear model (Table 4). By contrast, cohort 3 was defined by two modal components, while cohorts 4 and 5 by a single one. Therefore, for all three of these cohorts, we were unable to apply the F test, and a straight-line was accordingly fitted by default (Table 4; Fig. 5).

In the Coronda River, we constructed the length-frequency distributions using 7,290 mussels (Fig. 6). We identified three cohorts in each experimental stage (Fig. 7). The better model to describe the growth of cohorts 1 and 2 in the first stage was the linear one (Table 4). A straight-line was fitted by default to Cohort 3 and to the cohorts in the second stage since an F test could not be performed because of the low number of modal components (Table 4; Fig. 7).

The F test of the sum of squares between the linear models of growth fitted to the cohorts 1 and 2 of each study site gave significant differences ($F_{(2,9)} = 20.33$, $P < 0.001$ and $F_{(2,10)}$

$= 66.38$, $P < 0.001$; respectively). In both instances, the mussels from the Coronda River grew faster than those from the Santiago (Table 4; Fig. 8).

DISCUSSION

The present work constitutes the first comprehensive study analyzing and comparing the density, shell allometry, and growth of *L. fortunei* in dissimilar environmental conditions. Consistent with our predictions, the larval density, the density in settlement plates, and the growth of *L. fortunei* were significantly lower in the more polluted environment, the Santiago River. We also found significant differences in the shell allometry between the two populations of mussels. The individuals who exhibited a higher shell-growth rate and a higher population density in the less polluted environment were also more elongated (i.e., higher length-to-width ratio) than those from the more polluted habitat and lower density.

We observed a positive correlation between water temperature and larval density at both study sites. The larval density was very low or even nil at the end of autumn and the middle of winter, when the water temperature dropped

below 15–16°C at both locations. These results coincide with those of previous studies and would support the idea of a water-temperature threshold that modulated *L. fortunei* spawning events (Cataldo & Boltovskoy, 2000; Magara et al., 2001; Rojas Molina & José de Paggi, 2008; Boltovskoy et al., 2009a; Nakano et al., 2010). Moreover, the dissolved oxygen levels have been suggested to have a negative influence on the spawning and larval survival of this mollusk (Morton, 1977; Nakano et al., 2010; Oliveira et al., 2010b). We accordingly observed a strong negative correlation between the dissolved oxygen and the larval density in the Coronda River, but for reasons still unclear found no corresponding relationship in the Santiago River. We believe that this discrepancy might be associated with the low concentration and seasonal fluctuation in the dissolved oxygen that occurred mostly throughout the second year of the study.

The Santiago River has a higher average biological oxygen demand (9.2 mg/L; data provided by Engineering Department, La Plata Port Management Consortium) than the Coronda (4.3 mg/L and 2.3 mg/L during low and high water periods, respectively; Devercelli & Peruchet, 2008). These differing values of biological oxygen demand are fully consistent with the differences in dissolved oxygen estimated in this work, as the Santiago River's dissolved-oxygen concentrations were significantly lower than those of the Coronda River. The Santiago River exhibits heavy-metal concentrations some twenty- to fifty-fold above the recommended maximum levels for the protection of aquatic life (Steciow, 1998). The Río de La Plata estuary contains more than 300 point sources of pollution; and the anthropic input from domestic, industrial, and urban discharges have been identified as the primary cause of the eutrophication of this area (Kurucz et al., 1998). The level of pollution in the Santiago River, for its part, along with surrounding region, is significantly higher than in the Coronda River as a result of the input of hydrocarbon and heavy metals from nearby oil industries, the Río-Santiago shipyard, and the surrounding large urban centers (Colombo et al., 1989; Kurucz et al., 1998).

High concentrations of water pollutants and heavy metals can play a major role as stressors for aquatic animals and even have deleterious effects (Widdows & Donkin, 1992). *Limnoperna fortunei*, when exposed to heavy metals, manifests fertility disorders and a subsequent reduction in fecundity (Belaich et al., 2006).

Our results indicated that the larval density in the Santiago River, as the more polluted environment, was significantly lower than in the Coronda. Previous studies have looked at differences in *L. fortunei* larval densities in different aquatic ecosystems in the middle region of the Río de la Plata basin. For example, Rojas Molina & José de Paggi (2008) found differences between the Colastiné and the Santa Fe rivers ($17,200 \pm 3,200$ ind/m³ vs. $2,900 \pm 900$ ind/m³), but the authors provided no explanation for those regional differences. Boltovskoy et al. (2009a) also reported larval densities from different sites from the Río de la Plata basin and found that the densities from water bodies located close to the Coronda and Santiago rivers showed a variation ranging between about 4,000 and 7,000 ind/m³. These authors mentioned, however, that in other aquatic ecosystems, also from the Río de la Plata basin, the larval densities were far lower (450–729 ind/m³). They suggested that differences in larval densities were most probably a result of the size of the reproductive population settled at each site. In addition, Nakano et al. (2010) concluded that spatial differences in the larval density of *L. fortunei* between two lakes in Japan were the result of differences in the physicochemical environments of the two lakes. We believe that, despite a possible difference in the size of the reproductive population settled, the primary cause for the lower larval density observed in the Santiago River would be a biological response of mussels exposed to the higher level of pollutants at that site.

Variations in environmental conditions among sites clearly result in differences in mussel settlement, growth, and condition index (Dorgelo, 1992; Alfaro et al., 2008; Oliveira et al., 2011). Higher levels of pollution lead to higher larval-mortality rates and affect the settlement process by inhibiting the production of byssal threads (Widdows & Donkin, 1992). We accordingly found that the density of the settled individuals was significantly lower in the Santiago River – it being a more polluted environment than the Coronda. This result would corroborate the difference we observed in the larval densities. Indeed, the lower larval densities and higher levels of pollution in the Santiago River could have fully accounted for the decrement in the number of individuals in the settlement plates there. The population structure of the mussels in the Santiago River was best represented by individuals that were smaller than 6 mm in length, in contrast to the Coronda River, where the most representa-

tive individuals were larger than that value. Those structural differences were very likely related to differing growth rates *per se*, as the mussels in the Coronda River grew faster and reached larger sizes than the individuals in the Santiago.

Mollusks, like most organisms, exhibit progressive changes in their relative proportions with increasing body size as a response to high conspecific density dependence, as an ontogenetic process, or as a flexible consequence of environmental conditions (Alunno-Bruscia et al., 2001). Laterally compressed mussels grown at high densities would encounter fewer restrictions in valve opening than those compressed dorsoventrally (Lauzon-Guay et al., 2005). We found evidence that the mussels at higher population density in the less polluted environment of the Coronda River were significantly more elongated – that is, had a higher length-to-width ratio – than those at lower density in the more polluted environment of the Santiago. Nonetheless, we found no significant correlation between the density and the length-to-width values of the individuals in the Coronda River. When, however, we pooled the data from both sites, we found a positive relationship. We therefore favor the concept that mussels from high population densities tend to be more elongated – that is, longer – relative to individuals from low population densities (Lauzon-Guay et al., 2005). Based on these allometric relationships, we suggest that the observed differences in shell allometry could have also been related to a lower shell-growth rate in mussels from the more polluted environment.

Previous studies found similar growth rates of *L. fortunei* in Brazil (dos Santos et al., 2008), Asia (Morton, 1977; Iwasaki & Uryu, 1998; Magara et al., 2001), and in the Río de la Plata basin (Boltovskoy & Cataldo, 1999; Maroñas et al., 2003; Darrigran et al., 2011), but most of those investigations were restricted to specific locations and therefore lacked comparative analyses. Mussels living under different environmental conditions are likely to have different growth rates (Anthony et al., 2001). Bivalves exposed to habitats that are heavily polluted tend to bioaccumulate inorganic and organic contaminants (Widdows & Donkin, 1992). Consequently, such individuals exhibit poor growth and a decline in body weight that can negatively impact reproduction (Widdows & Donkin, 1992; Alfaro et al., 2008). Although a number of factors can affect growth, we hypothesized that the difference in environmental conditions between the present study sites could yield differences

in growth between the cohorts from the two sites. Our results demonstrated that the growth rates of the most representative cohorts from the Coronda River varied from 0.047 to 0.065 mm/day (i.e., 17–24 mm/year). By contrast, the growth rates of the most representative cohorts from the Santiago River were 0.026 to 0.035 mm/day (i.e., 10–12 mm/year). According to our prediction, mussels from the more polluted Santiago River grew more slowly than those from the less polluted Coronda.

We are aware that our study does not infer that polluted waters account directly for the differences we found in larval density, settlement, shell allometry, and growth in the two populations of mussels we sampled. In order to understand completely the causality of the differences we observed in the present study, experimental manipulations – both *in situ* and *in vitro* – of the multitude of factors that could conceivably affect the populations of *L. fortunei* would be needed. Such recourse would be both logistically difficult and highly time consuming. We believe, however, that this study has provided useful information on the potential effects pollution might have upon key bioecological aspects related to the life cycle of *L. fortunei*. Our results might therefore assist in the implementation of management protocols to prevent and/or diminish the ecologic and economic impact of nonnative species upon an ecosystem where that invader becomes established.

The invasive freshwater *L. fortunei* exhibits tolerance to a wide range of environmental conditions and can survive in many polluted water bodies with low pH, low oxygen, and low calcium concentrations where other invasive species (e.g., *D. polymorpha*) cannot (Karayayev et al., 2007). Our results, however, allow us to strongly suggest that the environmental conditions of heavily polluted environments might have a negative effect on larval density, the density in settlement plates, allometry, and the mussels' growth rates. Comparative studies are essential in the field of invasion ecology, because populations of nonindigenous species and their impacts are not homogeneous in either space or time (Boltovskoy et al., 2009a). An elucidation of the temporal and spatial population patterns of this bivalve will improve our understanding of its ecology, invasion dynamics, and impacts (Boltovskoy et al., 2009a). The evidence found in this work supports the idea that *L. fortunei* can inhabit heavily polluted environments that would be unsuitable for other invasive species, but nevertheless with a significant decrease in its biological potential.

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PHENOTYPIC DIFFERENTIATION OF ECOLOGICALLY SIGNIFICANT *BRACHIDONTES* SPECIES CO-OCCURRING IN INTERTIDAL MUSSEL BEDS FROM THE SOUTHWESTERN ATLANTIC

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ABSTRACT

Intertidal communities from rocky shores of the southwestern Atlantic are dominated by two small-sized mussels of the genus *Brachidontes*, *B. rodriguezii* (d'Orbigny, 1846) and *B. purpuratus* (Lamarck, 1819). Their generic placement, separation of specimens based on external conchological characters and geographic distribution are problematic. We conducted a comparative study based on extensive collections and observations at 14 sites along the coasts of the southwestern Atlantic, complemented by the study of materials from museum collections. Well-preserved specimens of the two species are distinguishable on the basis of shell characters, some of them previously ignored, over their combined latitudinal range.

Key words: *Brachidontes*, mussels, Mytilidae, southwestern Atlantic, rocky intertidal.

INTRODUCTION

Mid-intertidal communities from non-estuarine rocky shores of the southwestern Atlantic are dominated by densely packed populations of two small mussels belonging to the genus *Brachidontes* Swainson, 1840, namely *B. rodriguezii* (d'Orbigny, 1846) and *B. purpuratus* (Lamarck, 1819). Those mussel beds constitute the most conspicuous component among the intertidal molluscan-dominated assemblages in the region. The two species are ecologically significant ecosystem engineers, dominating the physiognomy and structure of mid-intertidal rocky shore communities (Adami et al., 2004) and increasing biodiversity through facilitation (Silliman et al., 2011).

The range of geographic distribution of *B. rodriguezii* is restricted to the warm-temperate Argentine Biogeographic Province (Balech & Ehrlich, 2008), whereas *B. purpuratus* occurs in the Magellanic Biogeographic Province (cold-temperate) and extends northwards along the southeastern Pacific to northern Peru (Coan & Valentich-Scott, 2012). Reported ranges overlap in northern Argentine Patagonia between approximately 41°S and 43°S.

The distinctness and phenotypic differentiation of these two ecologically significant species is still a matter of debate and much

confusion. Several authors have considered them to be synonymous (e.g., Aguirre et al., 2006), and even their generic placement remains debatable. They are often misidentified in ecological and palaeobiogeographic studies (e.g., Aguirre et al., 2006; Cuevas et al., 2006; Torres & Caille, 2009), and the boundaries of their distributions (Fig. 1) are not well resolved. This is due in part to them being phenotypically similar, which complicates identification particularly where their ranges overlap (Fig. 1). Also, mytilids are noted for their high phenotypic plasticity (Seed, 1968).

Here we describe both species on the basis of conchological characters. Shell characters indicated as diagnostic by previous authors, as well as features not considered before, were evaluated for consistency. In addition, we discuss their distributional range based on the literature, a survey of 14 sites along the Argentine coasts of the southwestern Atlantic, and examination of extensive museum collections, including existing type material.

MATERIAL AND METHODS

The study region extends over temperate and cold sectors of the southwestern Atlantic

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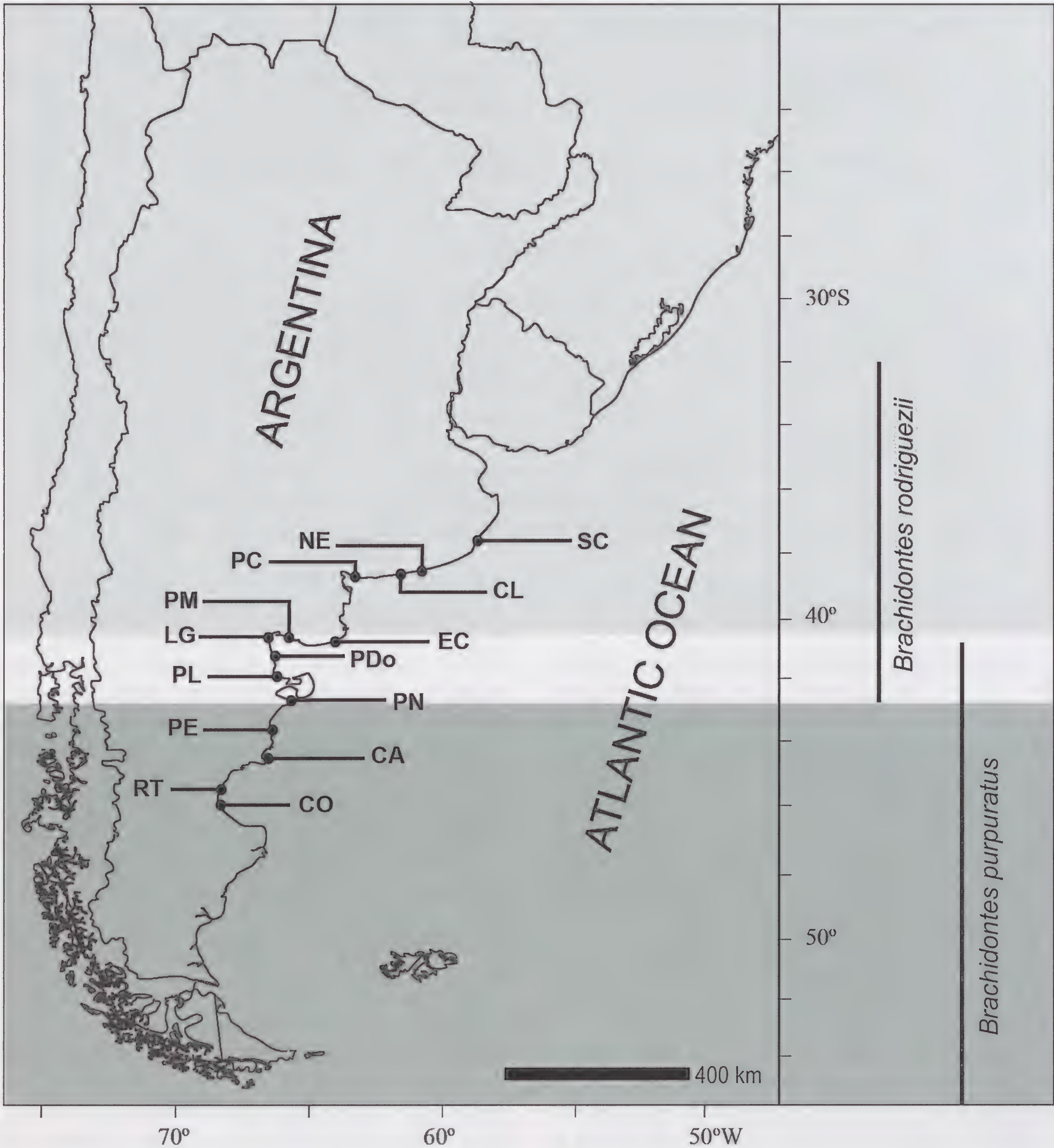


FIG. 1. Study region with indication of sampling survey sites, and latitudinal range of distribution of intertidal *Brachidontes* species within the region of interest. Abbreviations: SC: Santa Clara del Mar, NE: Necochea, CL: Claromecó, PC: Pehuencó, EC: El Cóndor, PM: Punta Mejillón, LG: Las Grutas, PDo: Playas Doradas, PL: Puerto Lobos, PN: Punta Ninfas, PE: Playa Escondida, CA: Camarones, RT: Rada Tilly, and CO: Caleta Olivia; Unshaded: Transition zone, light shading: Argentine biogeographic province, dark shading: Magellanic biogeographic province.

along the Argentine littoral (Fig. 1), known as Argentine and Magellanic provinces. The transition of both provinces for intertidal and nearshore habitats occurs in the gulfs San Matías, San José and Nuevo and the Valdés Peninsula (between 41°S and 43°S; Fig. 1).

Specimens from the mid-intertidal rocky zone were sampled at selected locations along the Argentine coast. Availability of candidate sites is limited by accessibility, particularly along stretches of the Patagonian shoreline. The survey included 14 sites over approximately

nine degrees of latitude from 37°S to 46°S. All samples were collected between 2004 and 2008. Voucher materials from all locations included in this study are housed at the Invertebrate Collection of the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia" (MACN) under the numbers MACN-In 37616-34. All *Brachidontes* specimens deposited in the collections of the MACN and Museo de La Plata (MLP) were also studied. In addition the lectotype of *Mytilus rodriguezii* d'Orbigny, 1846, designated by Aguirre (1994) and deposited at the Natural History Museum, London (NHMUK 1854.12.4.809/1), was re-examined and is here illustrated (Figs. 15–17). As was pointed out by Soot-Ryen (1955: 45), the type material of *Modiola purpurata* is missing. Y. Finet, and M. Huber from Geneve (pers. comm) confirmed that it is not at Museum d'Histoire Naturelle, Geneva, where most of the Lamarck's material is housed.

RESULTS

Family MYTILIDAE Rafinesque, 1815
Genus *Brachidontes* Swainson, 1840
Type species: *Modiola sulcata* Lamarck, 1819 (by monotypy)

Brachidontes rodriguezii (d'Orbigny, 1846)
Figs. 2–6

Mytilus rodriguezii d'Orbigny: 1846: 646; 1847: pl. 85, figs. 9–11.

Brachyodontes rodriguezii (d'Orb.): Castellanos, 1957: 8, pl. 2, fig. 7, pl. 4, fig. 8.

Brachyodontes rodriguezii (d'Orb.): Castellanos, 1970: 211, pl. 17, figs. 5, 6.

Brachidontes rodriguezii (d'Orbigny, 1846): Rios, 1994: 236, pl. 82, fig. 1164; Rios, 2009: 483, fig. 1357; Aguirre, 1994: 355, pl. 1, fig. 12a–d.

Brachidontes rodriguezii (d'Orbigny, 1846): Aguirre et al., 2006: 101, figs. 1, 5.



FIGS. 2–6. *Brachidontes rodriguezii* (d'Orbigny, 1846). FIGS. 2, 3: Claromecó, Buenos Aires Province, Argentina (MACN-In 37558); FIG. 2: Internal view of a right valve, arrows point to subterminal umbo and shell margin; FIG. 3: External view; FIGS. 4–6: Necochea, Buenos Aires Province, Argentina (MACN-In 37559); FIG. 4: External view of the left valve; FIG. 5: Dorsal view of articulated valves; FIG. 6: Internal view of the valve shown in Fig. 4; arrow points to the anterior adductor muscle scar. Scale bar = 5 mm.

Description: Shell mytiliform, thin, small to medium sized, approximately 40 mm length; umbones subterminal, rounded; marginal denticles posterior to ligament; hinge with 5–6 very small denticles. External ornamentation of radial, regular, very thin ribs, usually bifurcated, sometimes faint on the lower half; irregular growth lines covering the shell. Periostracum usually clear brownish on lower half of shell, and dark on the upper half. Interior dark reddish, iridescent.

Material Examined: (spm.: complete specimens, otherwise specified)

Uruguay: 34°02'38"S, 53°32'10"W, Punta del Diablo (MACN-In 37614), 20 spm.; La Coronilla (MLP 50023), 14 spm.; La Paloma, Rocha (MLP 4518-1), 3 valves; 34°40'03"S, 54°09'52"W, La Paloma (MACN-In 37615), 9 spm.

Argentina: Buenos Aires Province: Punta Canteras (MLP 7130), 100 spm.; Camet Norte (MLP 7139), 100 spm.; Miramar (MLP 7883), 1 spm.; Miramar (MLP 2323), 23 valves; 37°50'12"S, 57°29'46"W, Santa Clara del Mar (MACN-In 37616) 20 spm.; Mar del Plata (MLP 50037), 9 valves; Quequén (MLP 7132), 200 spm.; Necochea (MLP 2198), 2 valves; 38°37'20"S, 58°49'30"W, Necochea (MACN-In 37617) 20 spm.; Claromecó (MLP 7131), 200 spm.; 38°51'37"S, 60°03'28"W, Claromecó (MACN-In 37618) 20 spm.; Monte Hermoso (MLP 3615), 4 spm.; 39°00'12"S, 61°37'02"W, Pehuencó (MACN-In 37619), 20 spm.; Bahía Blanca (MLP 2692), 4 valves; Bahía Blanca (MLP 2855), 7 spm.; Bahía San Blas (MLP 4917-1), 10 valves; Río Negro Province: mouth of Río Colorado (MLP 1365), 7 valves; mouth of the Río Negro (MLP 50074), 1 spm.; 40°50'2"S, 65°05'07"W, Las Grutas (MACN-In 37620), 20 spm.; 41°03'28"S, 62°14'56"W, Balneario El Cóndor (MACN-In 37622) 20 spm.; Bahía Creek (MLP 4336-1), 132 spm.; 41°01'51"S, 64°08'34"W, Punta Mejillón (MACN-In 37623), 20 spm.; 41°37'41"S, 65°02'08"W, Playas Doradas (MACN-In 37625), 15 spm. Chubut Province: Punta Cantor, Península de Valdés (MLP 4133-2), 5 spm.; Playa Larralde, Golfo San José, Península Valdés, (MLP 7141-2), 18 spm.; Península Valdés (MLP 4316-3), 1 spm. Golfo San José, off Isla de Los Pájaros (MLP 4911-1), 5 spm.; Puerto Pirámides (MLP 5169), 2 valves; Golfo Nuevo (MLP 2859), 1 spm.;

Punta Cuevas, Puerto Madryn (MLP 13141), 9 spm.; Golfo Nuevo (MLP, 3720-1), 25 spm.; Golfo Nuevo (MLP, 2836-2), 12 spm.; Puerto Madryn (MLP 2399), 4 valves; 42°58'02"S, 64°19'01"W, Punta Ninfas (MACN-In 37628), 12 spm.

Distribution: From Rio Grande do Sul, Brazil (according to Rios, 2009), to Punta Ninfas, Chubut Province, Argentina (42°58'S). Cuevas et al. (2006) cited Caleta Olivia (47°26'S) as the southernmost locality, however we failed to find it in localities south of Punta Ninfas (42°58'02"S). Torres & Caille (2009) reported only *B. rodriguezii* for Puerto Madryn. We revisited their study sites and observed specimens of both species, *B. rodriguezii* and *B. purpuratus*, living together in the same beds.

Remarks: Aguirre (1994) designated as lectotype the specimen housed at NHMUK under the number 1854.12.4.809/1, which is illustrated here in Figures 15–17. This species has been dated as 1842. However, d'Orbigny's plate 85, while in a livraison with a cover date of 1842, did not appear until May 15, 1847. The species therefore dates from its appearance in d'Orbigny's 1846 text (N. Evenhuis, in preparation).

Brachidontes purpuratus (Lamarck, 1819)
Figs. 7–14

Modiola purpurata Lamarck, 1819: 113.

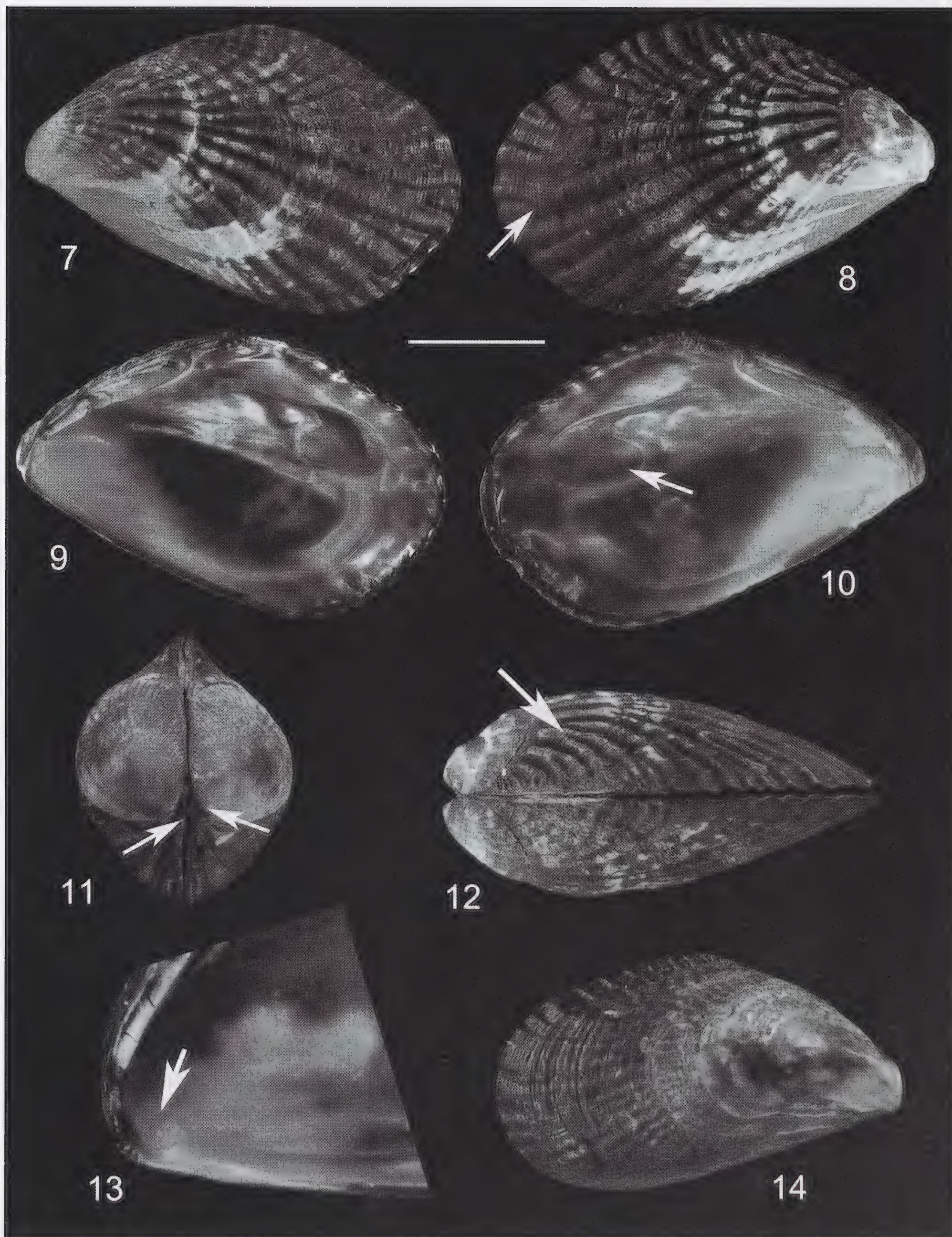
Brachidontes purpuratus (Lamarck) 1819: Soot-Ryen, 1955: 45, pl. 4, fig. 18, text fig. 30.

Brachyodontes purpuratus (Lamk): Castellanos, 1957: 8, pl. 2, fig. 7, pl. 4, fig. 8.

Perumytilus purpuratus (Lamarck, 1819): Olsson, 1961: 116. pl. 12, fig. 1, pl. 14, figs. 1, 1b; Osorio, 2002: 122, fig.; Coan & Valentich Scott, 2012: 119, pl. 37, fig.

Brachidontes purpuratus (Lamarck 1819): Huber, 2010: 117, fig.

Description: Shell small to medium sized, up to approximately 50 mm maximum length, subtriangular, ovate, thick, very variable; umbones terminal; sculpture of coarsely wrinkled radial, usually bifurcated, ribs. Hinge with several subequal denticles. Anterior adductor muscle scar thickened, raised, usually hyaline. Margin crenulated. Periostracum dark blue.



FIGS. 7–14. *Brachidontes purpuratus* (Lamarck, 1819). FIGS. 7–13: Camarones, Chubut Province, Argentina (MACN-In 37560); FIGS. 7, 10: External and internal view of a left valve; FIGS. 8, 9: External and internal view of a right valve, arrow points to the bifurcate rib and the posterior adductor muscle scar; FIG. 11: Umbo view, arrow points to the end of the umbo (right) and the shell (left); FIG. 12: Dorsal view of the same specimen, arrow points to a bifurcate rib; FIG. 13: Internal detail of the hinge area of a right valve; arrow points to the anterior adductor muscle scar; FIG. 14: Punta Ninfas, Chubut Province, Argentina (MACN-In 37561), right valve of a specimen showing intermediate characters. Scale bar for all specimens = 5 mm.



FIGS. 15–17. Lectotype of *Mytilus rodriguezii* d'Orbigny, 1846, NHMUK 1854.12.4.809/1; FIG. 15: External view of right valve; FIG. 16: External view of left valve; FIG. 17: Internal view of right valve. Scale bar for all specimens = 1 cm.

Material Examined:

Argentina: Río Negro Province: 40°50'02"S, 62°50'07"W, Las Grutas (MACN-In 37621), 8 spm.; 41°00'51"S, 64°11'34"W, Punta Mejillón (MACN-In 37624), 13 spm.; 41°36'41"S, 65°01'08"W, Playas Doradas (MACN-In

37626), 20 spm. Chubut Province: 41°59'10"S, 65°03'57"W, Puerto Lobos (MACN-In 37627), 20 spm.; Golfo San Matías, Península Valdés (MLP 4316-1), 36 spm.; Punta Cantor, Península Valdés (MLP 4133-1), 50 spm.; Puerto Pirámides (MLP 7406-1), 50 spm.;

TABLE 1. Comparison of morpological features of *Brachidontes rodriguezii* and *B. purpuratus*.

Character	<i>B. rodriguezii</i>	<i>B. purpuratus</i>
Valves	elongate (Figs. 2–6)	globular (Figs. 7–12)
Umbo	subterminal (Fig. 2)	terminal (Fig. 11)
Shell ribs	thin, smooth (Figs. 3, 5)	coarse (Figs. 7, 8, 12)
Rib pattern	parallel (Figs. 3, 4)	bifurcated (Figs. 7, 8, 12)
Adductor muscle scar	depressed, opaque (Fig. 6)	raised, hyaline (Fig. 13)

Playa Larralde, Golfo San José, Península Valdés (MLP 7141-1), 20 spm.; Golfo San José, off Isla de Los Pájaros (MLP 4911-2), 1 spm.; Golfo Nuevo (MLP 3720-3), 12 spm.; Punta Cuevas, Puerto Madryn, (MLP 13140), 9 spm.; 42°58'02"S, 64°19'01"W, Punta Ninfas (MACN-In 37629), 20 spm.; Punta Marques (MLP 1152), 35 valves; 43°43'21"S, 65°20'49"W, Playa Escondida (MACN-In 37630), 20 spm.; 44°54'34"S, 65°33'20"W, Camarones (MACN-In 37631), 13 spm.; 45°05'52"S, 66°30'26"W, Bahía Bustamante (MACN-In 37632), 20 spm.; 45°56'53"S, 67°33'12"W, Rada Tilly (MACN-In 37633), 19 spm.; Santa Cruz Province: Santa Cruz (MLP 2867), 5 valves; Comodoro Rivadavia (MLP 1711), 1 spm.; Comodoro Rivadavia (MLP 1704), 4 valves; Comodoro Rivadavia (MLP 5709), 20 spm.; 46°20'06"S, 67°34'14"W, Caleta Olivia (MACN-In 37634), 20 spm.; 47°44'36"S, 65°59'34"W, Puerto Deseado (MACN-In 37635), 20 spm.; Puerto Deseado (MLP 7884), 7 spm.; Puerto Deseado (MLP 3881), 6 spm.; Puerto Deseado (MLP 50028), 4 spm.; Puerto Deseado (MLP 4772-1), 2 spm.; Bahía del Fondo (MLP 1934), 23 spm.; Punta Piedra, Bahía San Julián (MLP 1708-1), 5 valves; 49°14'50"S, 67°40'12"W, Bahía San Julián (MACN-In 37636), 20 spm.; Bahía del Fondo (MLP 50021), 50 spm.; Bahía San Julián (MLP 50022-1), 22 spm.; B. Crosley, Isla de los Estados (MLP 50024-1), 15 spm.; Punta Peñas, San Julián (MLP 50025), 3 valves; Tierra del Fuego Province: Puerto Parry, Isla de los Estados (MLP 7384), 3 spm.; Isla Año Nuevo (MLP 4361-3), 1 spm.; Tierra del Fuego (MLP 1720) 3 valves; Tierra del Fuego (MLP 1721), 7 valves; Puerto Parry, Isla de los Estados (MLP 7384), 3 spm. Chile: Puerto Hundido (MLP 1723), 14 spm.; Punta Arenas (MLP 2353), 1 spm.; Puerto Montt (MLP 3680), 1 spm.

Distribution: The northernmost site where we recorded the presence of *B. purpuratus* is Punta Mejillón (41°S), northern coast of Golfo San Matías on the Atlantic Ocean. According to Coan & Valentich-Scott (2012: 119), this species occurs in the Pacific from Estero Zarumilla, Tumbes, Peru, to the Estrecho de Magallanes, Chile.

General Remarks

Valves of adult individuals are elongate in *B. rodriguezii* (Figs. 2–6), globular in *B. purpuratus* (Figs. 7–12). The position of the umbo is sub-terminal in *B. rodriguezii* (Fig. 2), but terminal in *B. purpuratus* (Fig. 11). Shell radial ribs are fine and smooth, closely spaced, with divarications not visible to the naked eye in *B. rodriguezii* (Figs. 3, 5), and coarse and divaricating, particularly on the anterodorsal and posterior margins, in *B. purpuratus* (Figs. 7, 8, 12). The scar of the anterior adductor muscle is depressed and opaque in *B. rodriguezii*, whereas it is raised and hyaline in *B. purpuratus* (Fig. 13). The confidence of identification increases with size, but can also deteriorate with shell wear in large individuals. In addition, some adult specimens (e.g., Fig. 14) are difficult to classify when only one character (usually the external ornamentation) is considered. Characters are comparatively summarized in Table 1.

DISCUSSION

We identified a series of characters that allow the unequivocal separation of the two species. Aguirre et al. (2006) used 2-D geometric morphometric techniques in an attempt to objectively separate three species of the genus *Brachidontes* [*B. rodriguezii*, *B. purpuratus* and *B. darwinianus* (d’Orbigny, 1846)], based

on planar projections of shell outlines. *Brachidontes darwinianus* was originally described from three locations: Rio de Janeiro (22°56'S, Brazil), Maldonado (34°55'S, Uruguay) and Ross Bay (41°09'S, Argentina). However, the record from Ross Bay probably corresponds to juvenile *B. rodriguezii*. Aguirre et al. (2006) could not find consistent differences and concluded that *B. darwinianus* and *B. rodriguezii* might be junior synonyms of *B. purpuratus*. Their pictorial illustration of phenotypic variation (Aguirre et al., 2006: fig. 4) is, however, difficult to interpret. Besides, none of the specimens illustrated resembles the type specimens of *B. rodriguezii* (Figs. 15–17). Some shells classified as *B. rodriguezii* by Aguirre et al. (2006) show diagnostic characters matching *B. purpuratus* (e.g., obviously bifurcate ribs). Their results, in our opinion, must be taken cautiously. *Brachidontes purpuratus* is the type species of the genus *Perumytilus* Olsson, 1961. According to Cox et al. (1969), the most comprehensive systematic compendium of bivalve genera, mussels belonging to *Brachidontes* Swainson, 1840, are defined as having “mytiliform shells with subterminal umbones, and radial sculpture on the ventral part simple or regularly bifurcating”. The genus *Perumytilus* (type species *Mytilus purpuratus* Lamarck, 1819, by original designation), however, was not mentioned by Cox et al. (1969). The genus is monotypic; the range of the single species mentioned by Olsson (1961) is Gulf of Guayaquil, Ecuador, to Chile. However, Soot-Ryen (1955) indicated that the range of this species is from the Straits of Magellan north in the Atlantic to Santa Cruz, Argentina. While the monotypic genus *Perumytilus* has been accepted by some authors in the taxonomic and ecological literature, Coan et al. (2010) and Huber (2010) considered *Perumytilus* to be a junior synonym of *Brachidontes*. More recently, Coan & Valentich Scott (2012: 119) used *Perumytilus* as a genus following its use by local authors. No new statement was included to support taxonomic change. Most of the primary characters considered to be of diagnostic significance are likely to show ecophenotypic variation. The taxonomic value of these features is open to discussion. Given that genus is a subjective category we prefer, for the time being, to keep both species studied here in the genus *Brachidontes*.

Olivier et al. (1966) reported only *B. purpuratus* in their ecological description of the rocky intertidal communities from Punta Pardelas,

Golfo Nuevo. Four decades later, Cuevas et al. (2006) indicated a mixture of both species at the same location. They concluded that the southern limit of *B. rodriguezii* in the southwestern Atlantic had expanded poleward to Caleta Olivia (47°26'S) during the intervening period. Unfortunately, there are no voucher materials were deposited to confirm these records. Our examination of samples from museums, including those collected by Olivier (1957) (MLP 3720), showed that both species have been present in Golfo Nuevo since at least the early 20th century. Specimens of both mytilid species were collected at Puerto Madryn in 1915 (MACN-In 9174-6, 9174-36) and 1924 (MLP 2399). Torres & Caille (2009) reported only *B. rodriguezii* from Puerto Madryn. In December 2009, we revisited their study sites and observed a mixture of the two species. We found no evidence of recent shifts in the range boundaries of *Brachidontes* spp. along the transition zone of the southwestern Atlantic, as suggested by previous authors. Kelaher et al. (2007) reported *B. granulatus* (Hanley, 1843) in the intertidal zone of Argentine Patagonia; apparently no voucher specimens from their study were kept (J. C. Castilla, personal communication). We did not find that species in our extensive survey (37°S to 46°S). *Brachidontes granulatus*, a very distinctive species, is distributed along the central and northern coasts of Chile, and it is not present on the Atlantic coast of South America. Two species of *Brachidontes* are known to occur along the Argentine coast; both are ecologically significant ecosystem engineers in the intertidal zone of rocky shores and other consolidated substrata. The morphological shell characters highlighted in our study are sufficient to differentiate them, and should be used to that end in surveys of biodiversity and experimental studies. This is of particular relevance where their latitudinal ranges of distribution overlap.

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REPRODUCTIVE BIOLOGY OF *ELEDONE CIRRHOSA* (CEPHALOPODA: OCTOPODIDAE) IN THE NORTHERN AND EASTERN TUNISIAN SEA (WESTERN AND CENTRAL MEDITERRANEAN)

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ABSTRACT

The reproductive biology of *Eledone cirrhosa* (Lamarck, 1798) was studied for the first time in the northern and eastern region of Tunisia. Monthly samples were provided from surveys carried out on board the Tunisian oceanographic vessel “Hannibal” and from commercial catches of bottom-trawl operating in this area from October 2005 to September 2006. Although specimens have been collected at a wide depth range, *E. cirrhosa* shows the greatest abundance between 100 and 300 m. The sex ratio was estimated at 0.94 with no significant difference ($P > 0.05$). Length-weight relationships calculated for each sex and the whole sample showed negative allometric growth ($b < 3$). The allometric coefficient in immature individuals revealed an allometric growth ($b < 3$) and isometric growth ($b = 3$) in maturing and mature individuals. A three-stage maturity scale was intended to distinguish between immature, maturing and fully mature animals. The reproductive season for females extended from April to August, with a spawning peak from June to August, and for males from January to August with a peak from April to August. The dorsal mantle length (DML) at maturity, corresponding to the size class when 50% of individuals were mature, was estimated at 6.88 cm in males, and 8.80 cm in females. The juveniles were mainly found in between 100 and 200 m where a nursery area for the species is probably present, while mature individuals were mostly fished at a depth greater than 200 m, which represents the spawning area of *Eledone cirrhosa*. Potential fecundity, estimated from 37 mature females, was 4171 ± 1216 oocytes. The number of spermatophores ranged from 42 to 121, with a mean length of 51.34 ± 4.86 mm.

Key words: *Eledone cirrhosa*, Tunisian northern and eastern coast, distribution, length-weight relationships, length at maturity, spawning season, fecundity.

INTRODUCTION

The horned octopus, *Eledone cirrhosa* (Lamarck, 1798), also named curled octopus, shows a wide geographic distribution, being found in the eastern Atlantic from the Moroccan coast to Iceland (Roper et al., 1984) and different parts of the Mediterranean Sea (the Tyrrhenian, Adriatic, Ionian, Aegean and the Thracian Sea) (Soro & Piccinetti-Manfrin, 1989; Wurtz et al., 1992; Lefkaditou & Papaconstantinou, 1995; Salman et al., 2000; Lefkaditou et al., 2003).

In the Mediterranean, the species inhabits muddy bottoms at circalittoral and bathyal levels (Mangold-Wirz, 1963), and its bathymetric distribution ranges between 30 and 500 m (Fisher et al., 1987) with greatest abundance

down to 300 m (Belcari et al., 2002). In the Tunisian waters, *Eledone cirrhosa* is a relatively abundant species and distributed essentially in the northern and eastern deep water where it is caught by bottom-trawl. *Eledone cirrhosa* is caught along Tunisian coasts by the trawl nets and it is mainly abundant in the northern and eastern waters on muddy, sandy and near the rocky bottoms.

Because of its great economic importance (Sánchez et al., 2004), *Eledone cirrhosa* has been the target of many specific studies both in North Atlantic (Boyle & Knobloch, 1982, 1983; Boyle, 1983) and in several areas of the Mediterranean; Catalan Sea (Mangold-Wirz, 1963), Gulf of Lion, France (Moriyasu, 1988), Ligurian Sea, Italy (Orsi Relini et al., 2006), and Aegean Sea (Tursi et al., 1995) but, there

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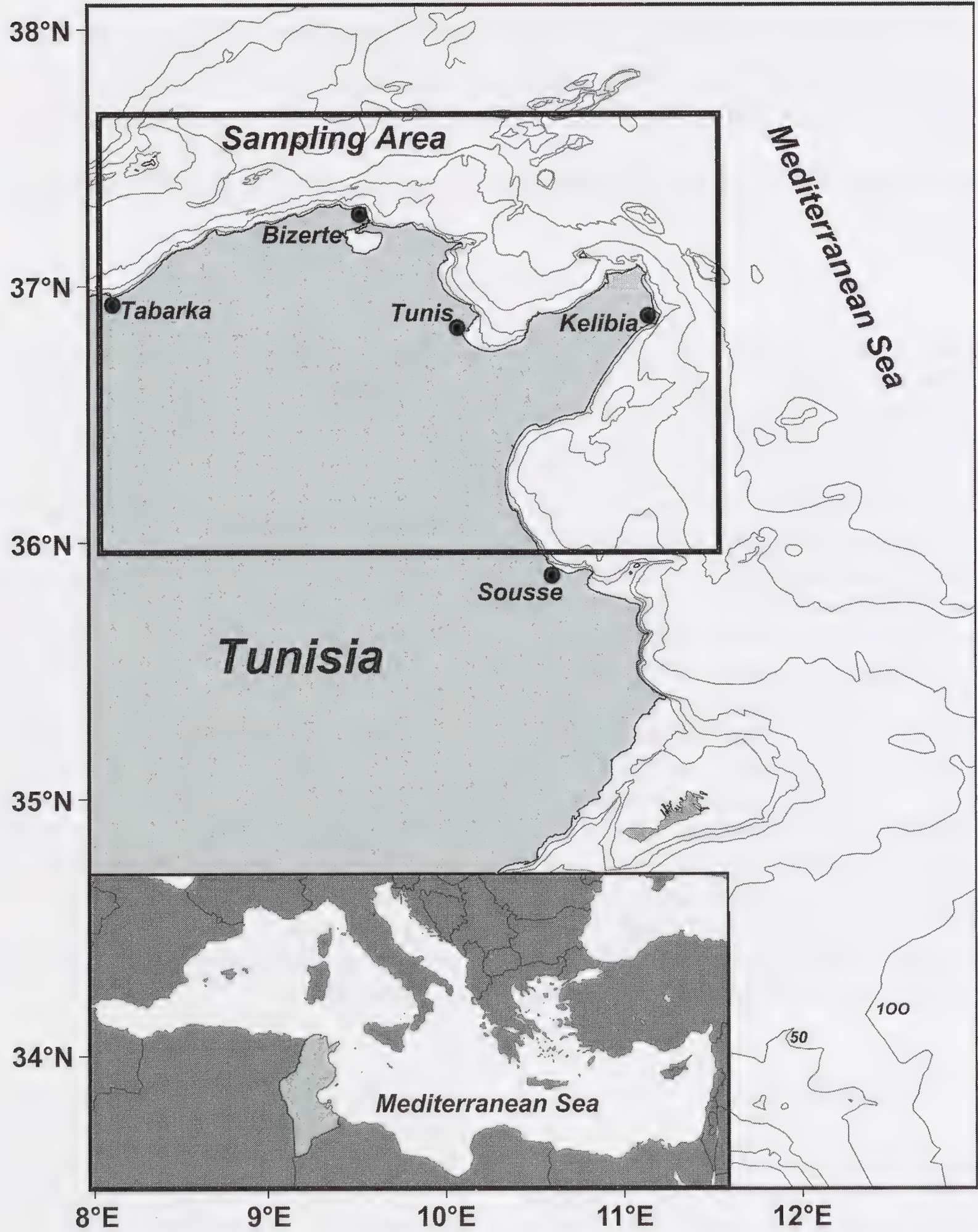


FIG. 1. Map of the study area.

have been no published studies on *Eledone cirrhosa* from Tunisian waters.

The aim of the present paper is to provide the first information of some basic aspects of *E. cirrhosa* from the northern and eastern Tunisian coasts (Fig. 1) to complement the information already obtained from others parts of the Mediterranean. The first detailed data on the reproduction biology related to the bathymetric distribution, season, length-weight relationships, sex ratio, size at maturity, spawning period and fecundity in the area are presented and discussed.

MATERIAL AND METHODS

The study area is the northern and eastern Tunisian waters limited in west by the Longitude 8°E and in the east by the 35°N latitude. *Eledone cirrhosa* was collected from surveys carried out on board the R/V “Hannibal” and from commercial bottom-trawlers from October 2005 to December 2006.

The adopted sampling is the random concept. In the fishing trade, *Eledone cirrhosa* is landed in bulk, without a previous prior sorting by size on board and it is exceptionally found in the

discards. In experimental surveys, the sampling is undertaken on whole catches. A total of 804 individuals randomly selected were sampled and analyzed for a detailed biological study. The dorsal mantle length (DML) and total body weight (BW) were measured and recorded in all specimens to nearest 0.1 cm and 0.1 g respectively. Specimens were dissected to determine sex and maturity stages. A three-stage maturity scale (adapted from Moriyasu, 1983) was used: (I) immature (in both sexes the gonad is very small, ovary and testis are transparent and membranous); (II) maturing (females with large reticulated eggs; males with more bulky testis and underdeveloped spermatophores); and (III) mature (female with large eggs ready to be spawned, the reticular system on their surface disappears; males with fully developed spermatophores for which a slight pressure on their spermatophoric sac discharges them through the penis to the mantle cavity). For the reproductive organs, the following weights in gram (g) were noted: Males, testis weight (TW) and spermatophoric complex weight (SCW); Females, ovary weight (OW) and oviductal complex weight (OCW) (oviduct + the oviductal gland); Sex and maturity stages were recorded.

The bathymetric distribution by depth strata of the species was described from the results obtained in the scientific bottom-trawl surveys series carried out in the framework of the INSTM (National Institute of Marine Sciences and Technologies) program in the northern (20–800 m) and eastern Tunisian coasts (20–320 m) in 2005 and 2006. The surveys were carried out with a GOV55/42 trawl gear equipped with a 20 mm mesh cod end liner and the bottom trawl. The sampling hauls were distributed according to a stratified sampling scheme, with random drawing inside each stratum. Five-depth strata were identified: 50–100, 100–200, 200–300, 300–400 and deeper than 400 m, but we considered in this work only four strata because in the horned octopus occurred in small numbers below 300 m. To detect size changes in terms of depths, mean sizes of *E. cirrhosa* by stratum were calculated and differences compared using the Student's t-test. Bathymetric distribution was also studied by season and maturity stages.

Length-weight relationships (DML-BW) were estimated for the species, by sex and according to the maturity stage. Equations of the form $BW = a \cdot DML^b$, where a and b , constants of regression, were fitted by log transforming the data

and deriving the regression line by the least squares method. An analysis of covariance (ANCOVA) was used to test the differences between the slopes obtained for each sex. A Student's t-test was applied to evaluate relative growth isometry.

The sex-ratio was calculated considering the whole data set, by month and in relation to body size-ranges. The significant differences between sex-ratios were analyzed by means of the chi square test (χ^2 -test).

The dorsal mantle length at maturity ($DML_{50\%}$) was estimated by fitting the relative length-frequency distribution of mature individuals (stage III) to a logistic model as described by the following equation:

$P = 1/(1 + \exp(-a(DML - DML_{50\%})))$ (King, 1995), where P is the relative frequency of mature individuals per length class (DML) and a is the slope parameter of the maturity curve.

The spawning season was estimated by analyzing monthly changes in the values of the maturity indices and in the proportions of the advanced maturity stages throughout the year. Indices of reproductive status for male and female were calculated using the following expressions:

The gonadosomatic index (GSI):
 $GSI = (GW/EW) \cdot 100$, with: GW: gonad weight, EW: eviscerated body weight (digestive and reproductive organs were removed).

Guerra's index (GI) (Guerra, 1975) for male: $GI = SCW/(SCW + TW)$; for female $GI = OCW/(OCW + OW)$.

Fecundity was studied in 37 mature females ranged and weighted respectively from 8 cm to 15 cm and from 82.31 to 869.21 g. Potential fecundity (PF) was calculated as the total number of oocytes of all sizes present in the ovary (Laptikhovsky, 1999). Relative fecundity (RF) was estimated as the ratio of PF to BW. Size of oocytes were measured to the nearest 0.01 mm. Spermatophores from 75 mature males were also counted and measured to the nearest 0.01 mm.

RESULTS

Bathymetric Distribution

A total of 250 bottom-trawling hauls, undertaken by commercial and research surveys throughout all seasons of the year and in a depth ranging from 20 to 800 m, were analyzed (Table 1). This sampling yielded a total of 698

TABLE 1. Bathymetric distribution of *E. cirrhosa* by stratum and by sex. FDML: mean female dorsal mantle length; MDML: mean male dorsal mantle length; (M+F)DML: female and male mean mantle length; NF: female number; NM: male number; N(M+F): female and male number; %M+F: female and male proportion.

Stratum (m)	FDML (cm)	NF	% F	MDML (cm)	NM	% M	(M + F) DML (cm)	N (M + F)	% M + F
50–100	9.08	9	81.80	8.50	2	18.20	8.97	11	1.60
100–200	9.50	204	58.00	8.32	148	42.00	9.00	352	50.40
200–300	9.29	146	49.20	8.77	151	50.80	9.03	297	42.60
> 300	10.81	17	44.70	9.20	21	55.30	9.92	38	5.40
Total	9.47	376	53.90	8.59	322	46.10	9.06	698	100

individuals (322 males and 376 females), ranging from 4 to 13 cm DML in males and from 3 to 16.5 cm DML in females.

Eledone cirrhosa showed a wide distribution. According to bottom-trawl surveys (Table 1),

its presence was recorded from 50 to 580 m, but catches were sporadic in water in bottoms shallower than 100 m (1.6%) and deeper than 300 m (5.4%). The best yield (93%) was located between 100 m and 300 m.

TABLE 2. Bathymetric distribution of *E. cirrhosa* males by season and maturity stage. MDL: Mean Dorsal Mantle Length (cm); N: total Number by stratum; NIM: Number of immature individuals; NM: number of mature individuals; % M: % of mature individuals number; significant difference for underlined numbers $P < 0.05$.

Season	Stratum (m)	MDML per stratum	N	$P < 0.05$	NIM	NM	% M	MDML per season $P < 0.05$
Autumn	50–100	0	0		0	0	0	8.45
Autumn	101–200	<u>6.39</u>	59		47	12	20.3	
Autumn	201–300	<u>8.20</u>	57	$4.98e-12$	38	19	33.3	
Autumn	> 300	0	0		0	0	0	<u>7.96</u> 0.37
Winter	50–100	0	0		0	0	0	
Winter	101–200	<u>10.86</u>	7		0	7	100	
Winter	201–300	<u>7.44</u>	5	$4.78e-06$	0	5	100	
Winter	> 300	0	0		0	0	0	
Spring	50–100	0	0		0	0	0	<u>9.16</u> 0.01
Spring	101–200	<u>8.99</u>	40		1	39	97.5	
Spring	201–300	<u>9.74</u>	76	0.0023	3	73	96.1	
Spring	> 300	9.20	21	0.11	0	21	100	
Summer	50–100	8.50	2		0	2	100	<u>7.74</u> $6.71e-08$
Summer	101–200	<u>9.96</u>	42	0.41	10	32	76.2	
Summer	201–300	<u>6.11</u>	13	$2.82e-09$	10	3	23.1	
Summer	> 300	0	0		0	0	0	
All seasons	50	8.50	2		0	2	100	
All seasons	101–200	<u>8.32</u>	148	0.91	58	90	60.8	
All seasons	201–300	<u>8.77</u>	151	0.03	51	100	66.2	
All seasons	> 300	9.20	21	0.33	0	21	100	

TABLE 3. Bathymetric distribution of *E. cirrhosa* females by season and maturity stage. MDML: Mean Dorsal Length (cm); N: total Number by stratum; NIM: Number of immature individuals; NM: number of mature individuals; %M: % of mature individuals number; significant difference for underlined numbers $P < 0.05$.

Season	Stratum (m)	MDML per stratum	$P < 0.05$	NIM	NM	% M	NDML per season	$P < 0.05$
Autumn	50–100	0		0	0	0	<u>7.51</u>	
Autumn	101–200	<u>7.02</u>		57	2	3.4		
Autumn	201–300	<u>7.92</u>	0.007	35	1	2.8		
Autumn	> 300	0		0	0	0		
Winter	50–100	<u>5.45</u>		2	0	0	<u>8.36</u>	0.02
Winter	101–200	<u>10.80</u>	4.90e-05	15	0	0		
Winter	201–300	<u>7.08</u>	1.77e-10	11	1	8.3		
Winter	> 300	0		0	0	0		
Spring	50–100	10.76		1	4	80	<u>10.46</u>	3.50e-11
Spring	101–200	<u>9.70</u>	0.15	2	75	97.4		
Spring	201–300	<u>10.77</u>	5.78e-06	12	70	85.4		
Spring	> 300	10.81	0.93	0	17	100		
Summer	50–100	8.50		0	2	100	<u>9.64</u>	0.007
Summer	101–200	<u>11.61</u>	0.14	9	44	83		
Summer	201–300	<u>6.44</u>	1.84e-11	13	3	18.8		
Summer	> 300	0		0	0	0		
All seasons	50–100	9.08		3	6	66.7	8.40	
All seasons	101–200	9.50	0.64	75	119	58.3		
All seasons	201–300	<u>9.29</u>	0.38	60	88	60.3		
All seasons	> 300	<u>10.81</u>	0.01	0	17	100		

TABLE 4. Length-total weight relationships of *E. cirrhosa* from the northern and eastern region of Tunisia by sex and maturity stage (immature and maturing/mature). N: number of individuals; DML: mean mantle length; SD: standard deviation; R: correlation coefficient; a and b: parameters; s: significance level of b compared to 3 ($*P < 0.05$, ns, not significant).

	Sex	N	DML range (cm)	DML $\pm$ SD	BW range (g)	BW $\pm$ SD
Total	M	414	4–12.5	78.64 $\pm$ 17.72	14.54–517.36	113.42 $\pm$ 68.41
	F	390	3–16.5	83.99 $\pm$ 23.74	12.46–869.21	149.80 $\pm$ 125.61
Immature	M	116	4–10.2	7.49 $\pm$ 1.38	14.54–205.25	89.55 $\pm$ 41.65
	F	227	3–10.5	6.97 $\pm$ 1.28	12.46–222.44	75.64 $\pm$ 38.23
Maturing/mature	M	298	4–12.5	8.05 $\pm$ 1.91	17.14–517.36	127.20 $\pm$ 79.94
	F	163	6.5–16.5	10.81 $\pm$ 1.76	59.73–869.21	275.62 $\pm$ 121.55

The bathymetric distribution seems to vary with sex, season and maturation stage.

Distribution by Sex

In stratum 50–100 m, 9 females among 376 sampled and 2 males on 322 were counted (Table 1); females were significantly more represented (81.8%) than males. Both strata 100–200 and 200–300 m were the most frequented in considering sex (93% of total sampled individuals). Sex-ratio was almost equal to 1:1. In the Catalan Sea, Mangold-Wirz (1963) confirmed that beyond 250 m, *E. cirrhosa* were collected equally but always in very small quantities.

Distribution per Season and Maturity

In autumn, few males were mature, their proportion did not exceed 33% in the strata between 100 and 300 m (Table 2). In winter and the seasons that follow, the majority became mature, with a continuing migration toward the coast. However, almost all females were immature in autumn and winter (Table 3); they reached the maturity in spring and summer and seemed to occupy all strata.

Length-Weight Relationships

The overall length-total weight relationships of the studied population of *E. cirrhosa* in the northern and eastern sea of Tunisia can be described by the following expression: $BW = 0.3283 \times DML^{2.7706}$ ($r = 0.9118$)

The value of the *b* coefficient (< 3) indicates that this species shows overall negative allo-

TABLE 6. Sex ratio of *E. cirrhosa* from the Northern and Eastern coast of Tunisia by month and the total sample (*-significant difference (χ^2 -test, $P < 0.05$)). Nm: number of males; Nf: number of females; Nt: total number of males and females; m/f: male/female ratio.

Month	Nm	Nf	Nt	m/f	χ^2
October	76	66	142	1.15	0.70
November	45	63	108	0.71	3.00
December	43	38	81	1.13	0.31
January	28	26	54	1.08	0.07
February	39	24	63	1.63	3.57
March	32	25	57	1.28	0.86
April	22	27	49	0.81	0.51
May	15	38	53	0.39	9.98*
June	19	28	47	0.68	1.72
July	23	26	49	0.88	0.18
August	18	24	42	0.75	0.86
September	30	29	59	1.03	0.02
Total	390	414	804	0.94	0.72

metric growth. The same results were obtained when males and females were analysed separately (Table 4). There was no significant difference when the slopes of the power formula were compared between the two sexes (ANCOVA, $F_{1, 800} = 0.327$, $P = 0.567$). In immature individuals *b* values were significantly less than 3 for males and females, while maturing and mature specimens of both sexes showed isometric growth ($b \sim 3$) (Table 4). When the slopes were compared between sexes with the respective values of length-total weight relationships, a significant difference was observed in maturing/mature (ANCOVA, $F_{1, 457} = 55.11$, $P < 0.005$). Thus, at the same size, slight superiority in weight is manifested in females due to an important increase in gonad weight in relation to those of males.

Length-somatic weight relationships showed negative allometric growth for the total sample and also for immature and maturing/mature individuals (Table 5). When slopes were compared with the respective values of length-total weight relationships, a significant difference was observed in females (ANCOVA, $F_{1, 322} = 5.556$, $P = 0.1914$) and a faster relative growth in the reproductive organs as a consequence of the maturation process; while for maturing/mature males no significant difference in the slopes was found (ANCOVA, $F_{1, 592} = 0.1030$, $P = 0.748$).

TABLE 5. Length-somatic weight relationships of *E. cirrhosa* from the northern and eastern region of Tunisia by sex and maturity stage. *r*: correlation coefficient; *a* and *b*: parameters; *s*: significance level of *b* compared to 3 (* $P < 0.05$).

	Sex	N	R	a	b	S
Total	M	414	0.8778	0.3139	2.7121	*
	F	390	0.9235	0.3487	2.6773	*
	M + F	804	0.9051	0.3292	2.6972	*
Immature	M	116	0.727	0.608	2.357	*
	F	227	0.756	0.570	2.404	*
Maturing/ mature	M	298	0.89	0.312	2.732	*
	F	163	0.73	1.149	2.196	*

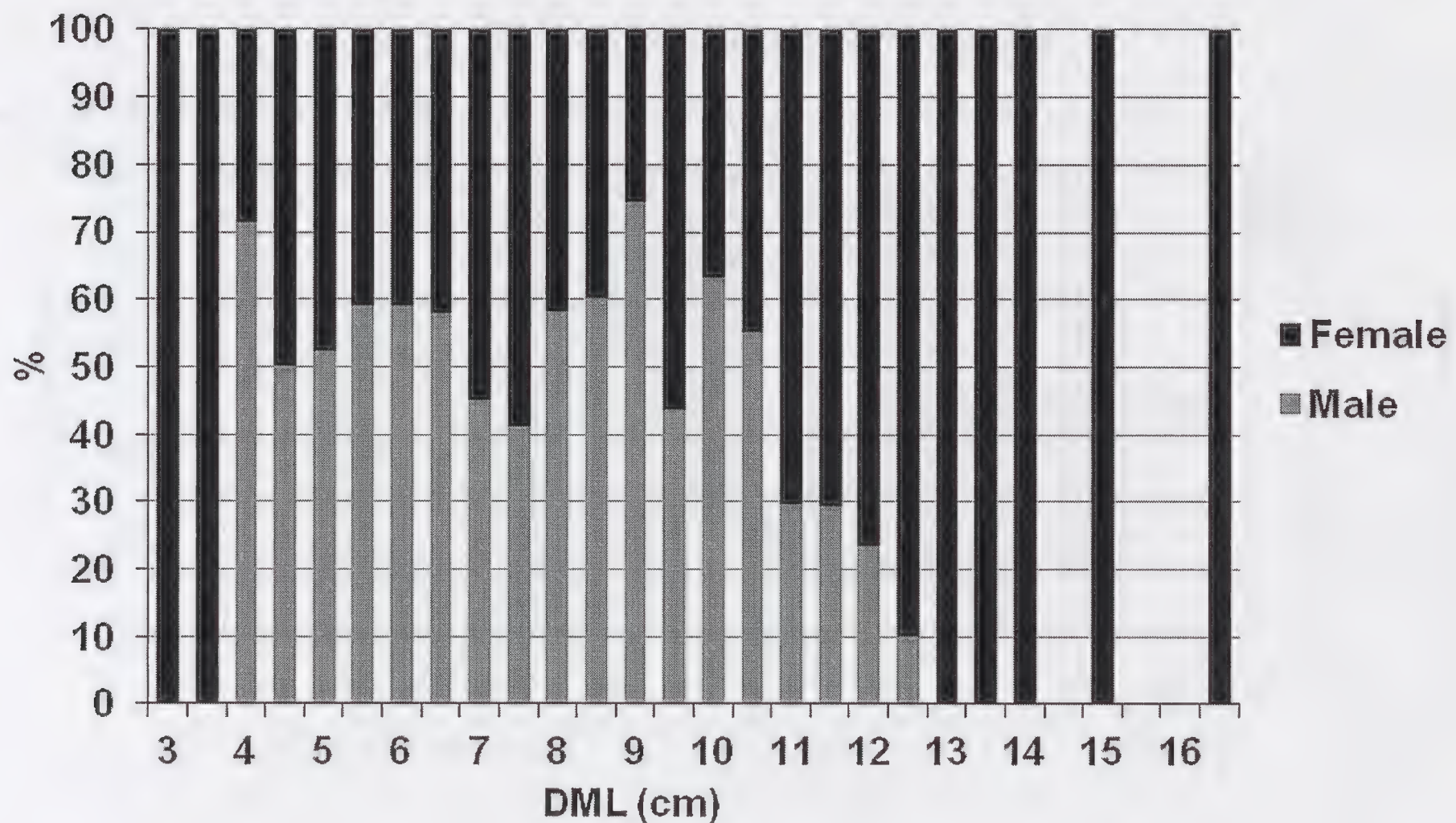


FIG. 2. Sex-ratio in terms of class-size in *Eledone cirrhosa*.

Sex Ratio

Of the individuals examined, 414 were females and 390 were males. The overall ratio of males to females was 0.94:1. The application of the χ^2 -test showed no significant difference ($P > 0.05$) even when analyzing the sex ratio by month except for May when there is a significant difference between sexes (0.39:1; $P < 0.05$) (Table 6). The number of males and females in length class ranged between 3 to 10.5 cm was not significantly different ($P > 0.05$). More females than males in DML classes from 11 cm onwards were recorded (Fig. 2).

Size at Maturity

The mantle length of mature individuals ranged between 5.8 and 12.5 cm in males and between 6.5 and 16.5 cm in females. The estimated mean size at which 50% of males were mature was 6.88 cm (Fig. 3A), whereas the estimated mean size at which 50% of females were mature was 8.80 cm (Fig. 3B).

Spawning Season

The monthly evolution of the maturity stages in males and females during the sampled period is represented in Figure 4. Immature males were found throughout the year, except in June and July. Mature males were observed from January to July 2006, showing a reproductive

activity in this period; the maximum maturity was reached in June–July since all sampled males were mature.

The monthly distribution of the maturity percentages in female *E. cirrhosa* follows the same pattern as in males. Nevertheless, the male maturity seems to be more precocious since beginning in November their maturing rate exceeded 50%, while the females were yet scarcely immature (Fig. 4). During the sampling period, two different stages may be defined: (a) in autumn–winter, when the percentage of immature females was higher than that of mature females; (b) in spring–summer, when an increase in the percentage of mature females was observed to reach 100% in July, which coincides with the observed peak in maturity. The reproductive peak in females coincides with that of males in July and August.

The maturity indices of males and females show a similar trend to that of the proportion of maturity stages over the year. Figure 5 shows the monthly evolution of the GSI for both sexes.

Females have their minimum values from October 2005 to January 2006. Since then, there is an increasing trend until the spring of the following year. In June, a higher peak is observed, followed by a decrease in July corresponding to the spawning period. In males, the graphical representation of GSI (Fig. 5) shows an important period corresponding to the mature stage from December to June; the

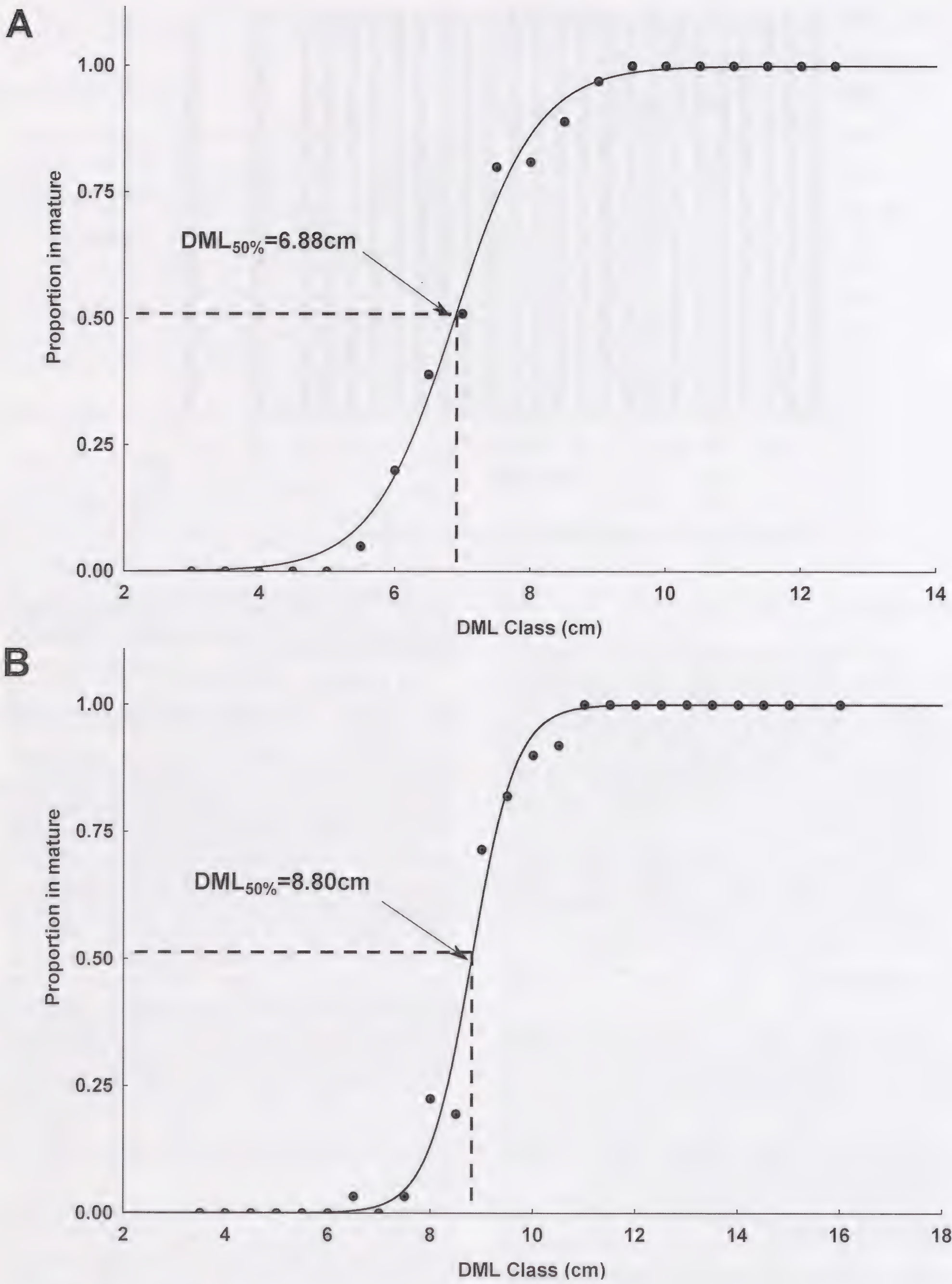


FIG. 3. Size at maturity for males (A) and females (B).

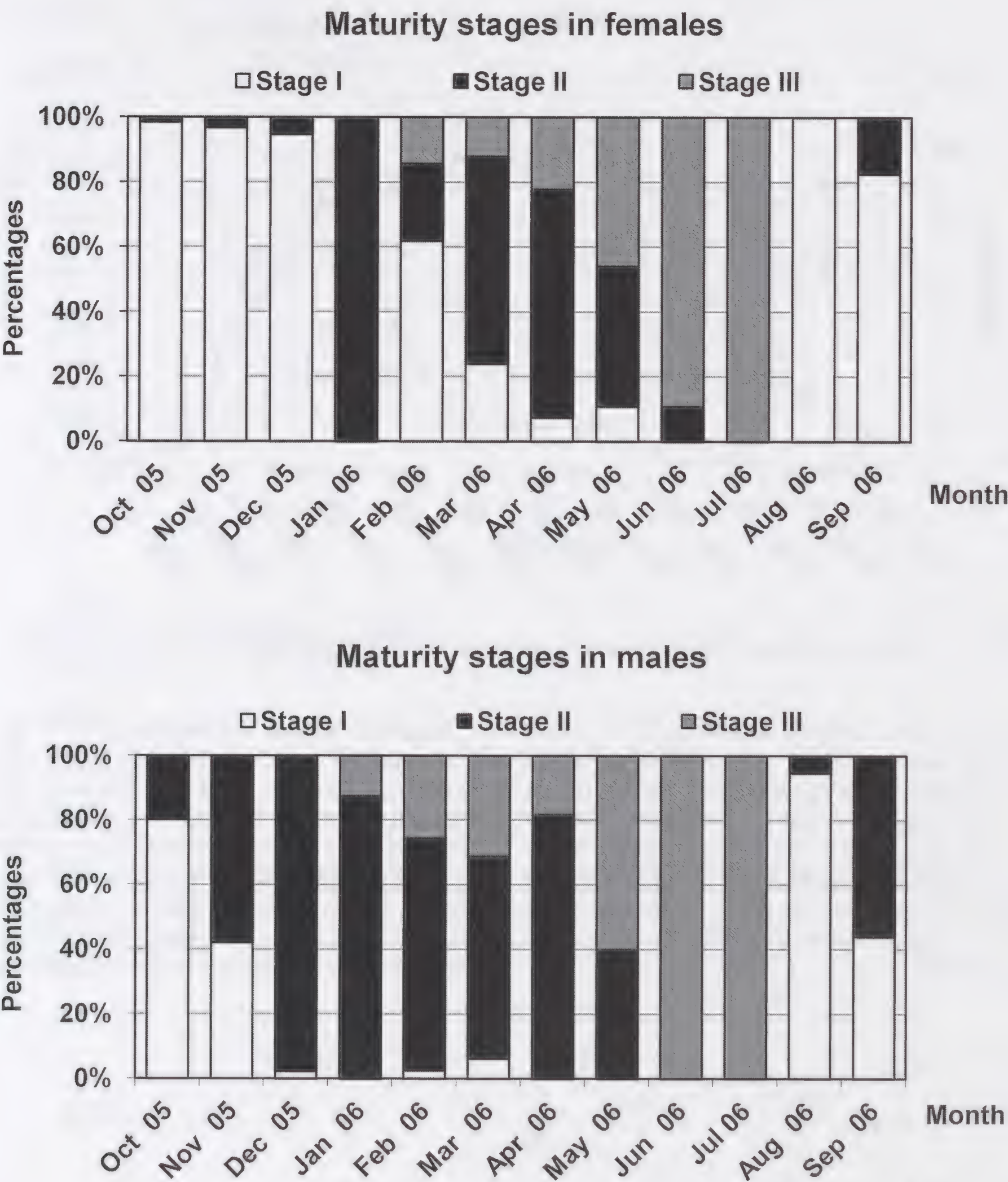


FIG. 4. *Eledone cirrhosa*. Monthly percentages of maturity stage (I–III) in females and males.

spermatophore emission period initiated in July coincides with the egg laying in females.

The GI index is represented in Figure 6, for both sexes. The females show minimum values in spring and summer, coinciding with the months where the proportion of mature females and maximum GSI values were higher. In males, the highest values are reached in the months when the greater proportion of mature males was elevated, that is, from April to July. During sexual maturation, the spermatozoa produced in the testis go through the tract

and are stored inside the spermatophores in Needham’s complex. The continuous transfer of spermatozoa induces an increasing of the tract weight which becomes clearly higher than that of the testis and therefore an increase in the maturation index, reaching its maximum at full maturity (Najai, 1983).

Fecundity

The number of oocytes in the ovaries of mature females ranged from 1859 in a female

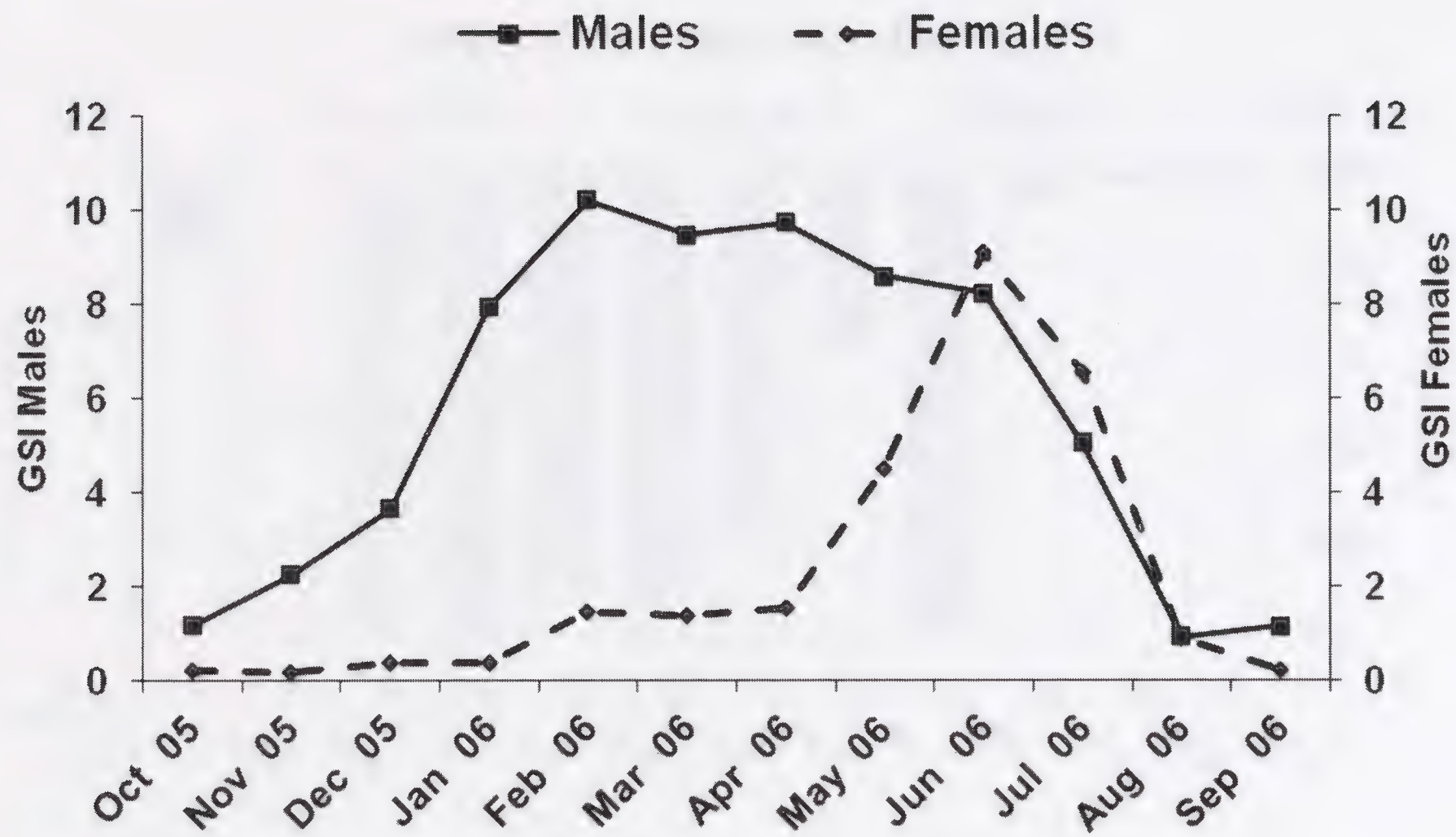


FIG. 5. *Eledone cirrhosa*. Monthly evolution of the gonadosomatic index in males and females.

of 10.5 cm DML, a body weight of 231.8 g and an ovary weight of 3.84 g, to 8500 in a female of 14 cm DML, a body weight of 869.21 g and an ovary weight of 79.65 g. The mean potential fecundity was 4171 ± 1216 oocytes per female. The average relative fecundity, defined as the number of oocytes per gram of female, was 11.88 ± 4.54 oocytes per gram of the total body weight.

Figure 7 shows the relationship in females between the potential fecundity in terms of size (DML) and ovary weight (OW). There was a weak relationship between the potential fecundity and the mantle length of mature females ($r = 0.67$; $P < 0.001$) even though there was a tendency for the larger females to be more fecund than smaller ones. The weak relationship may result from some females of similar size having

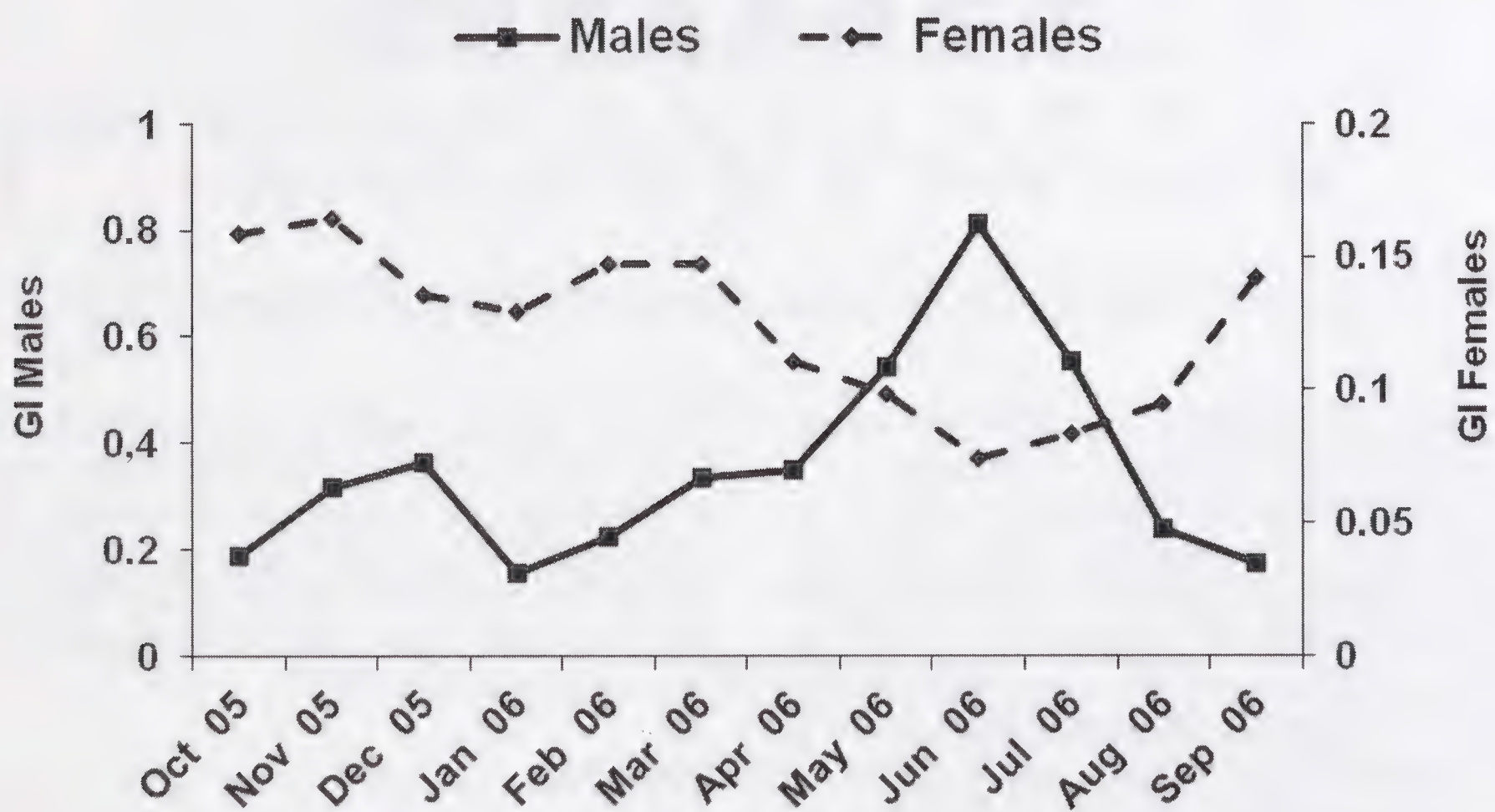
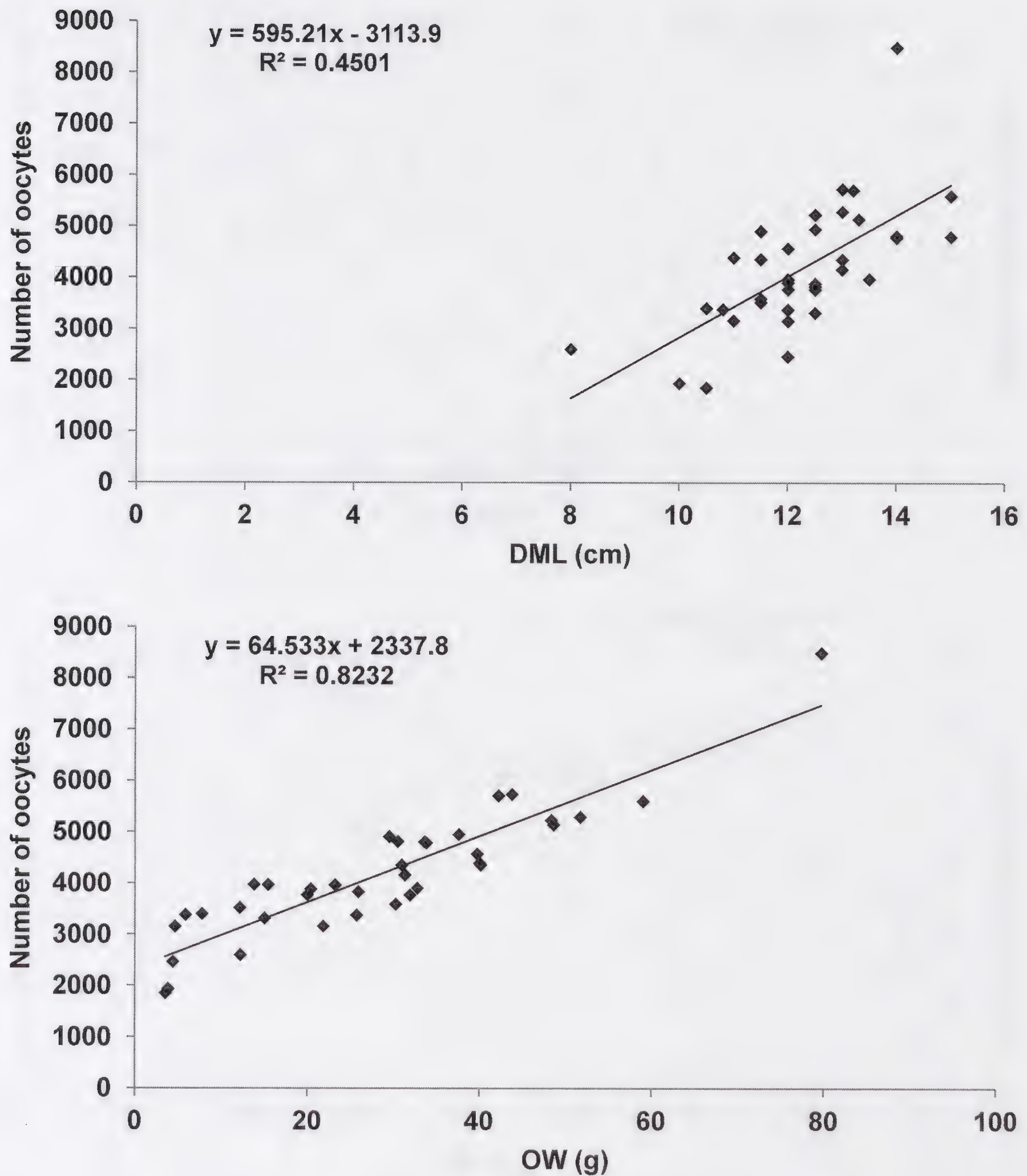


FIG. 6. *Eledone cirrhosa*. Monthly evolution of the maturation index in males and females.



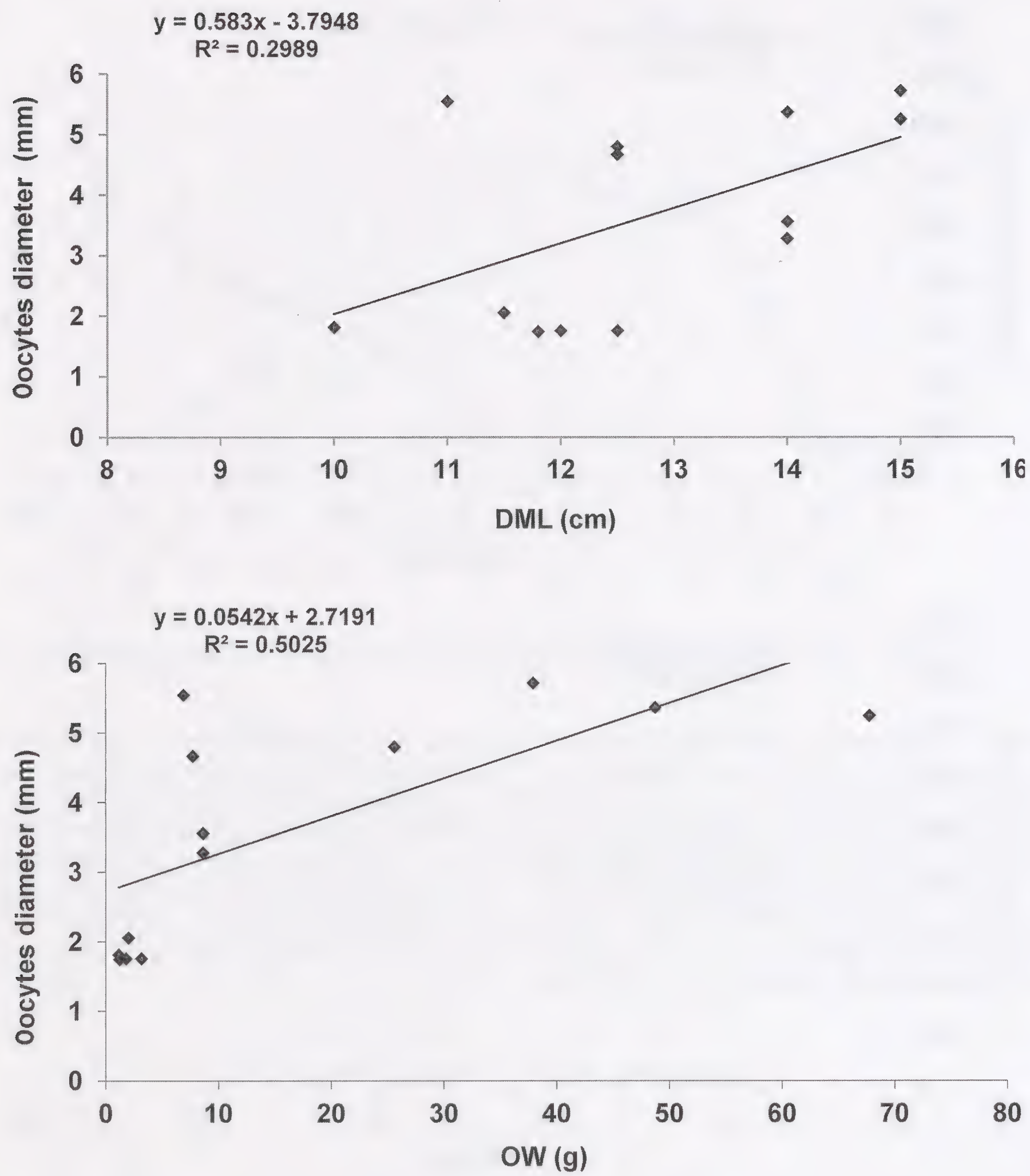


FIG. 8. Relationship between diameter of oocytes and dorsal mantle length (DML), and ovary weight (OW) in the female of *Eledone cirrhosa*.

0.001) (Fig. 9). The wide scatter was expected because some males had already released a proportion of their spermatophores and all these individuals appeared to be in spawning condition.

The length of spermatophores varied between 40 mm in a male measuring 6.8 cm and 59 mm in a male measuring 12 cm (mean = 51.34 mm, SD = 4.86, N = 70). A positive correlation was found between the mantle length and the length of spermatophores ($r = 0.88$; $P < 0.001$) (Fig. 9).

DISCUSSION

The scientific surveys taken in northern and eastern Tunisian sea showed no preference for any particular zone within the investigated area, but we noted the best yield obtained over the grounds where crustacean species were abundant, especially *Parapeaneus longirostris*. Also, in the northern North Sea and waters to the west of Scotland over which *E. cirrhosa* occurs are equally rich in crustacean species (Boyle, 1983).

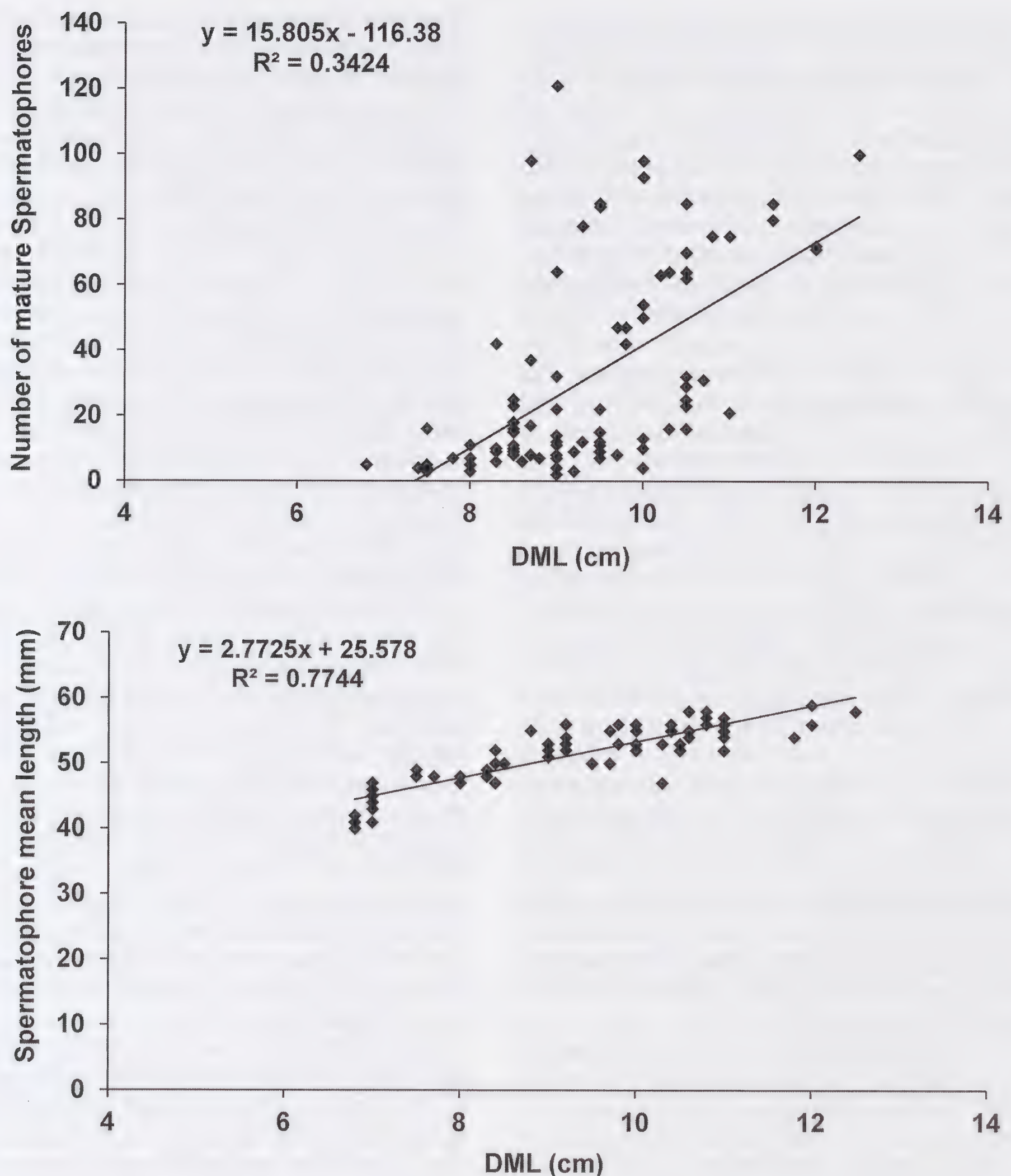


FIG. 9. Relationship between dorsal mantle length (DML) and number of mature spermatophores, and mean length of spermatophores in the male of *Eledone cirrhosa*.

The bathymetric distribution of *E. cirrhosa* observed in the northern and eastern Tunisian waters confirm that reported for other Mediterranean areas. The species inhabits a wide range of bottom depths, from 50 to 580 m, even though it is usually concentrated in the first 300 m (Salman et al., 2000; Belcari et al., 2002). The presence of specimens beyond 500 m has also been reported along the Italian coast (Wurtz et al., 1992; Relini et al., 1999).

On the basis of the comparison of mean size in terms of depth and season, males and females

taken together, recruits were mainly found in the 200–300 m stratum in the late summer especially in August. Almost all specimens caught in this deep were immature (DML ranging between 3 and 6.7 cm). Juveniles were fished exclusively deeper than 100 m in autumn and winter. Adult individuals were found at depths greater than 100 m and mature ones mostly beyond 200 m in spring. The disappearance of these mature individuals from catches in summer suggests that these animals could be spawners inhabited the hard substrata where eggs would be laid.

According to Mangold-Wirz (1963) such hard substrata are found on the shelf: according to the opinions of other authors, on the slope. In our study area, the depths greater than 200 m are characterized by restricted trawling because of extensive rocky surface (Castany, 1955; Azouz, 1974). This fact suggests that these bathyal hard substrata are the most probable egg-laying areas. These results confirm those reported by Relini et al. (2006) in the Ligurian Sea, thus the depth ranging between 100 and 200 m could represent a nursery area for *E. cirrhosa* in the northern and eastern sea of Tunisia while the deeper grounds (> 200 m) might constitute a spawning area for this octopus.

In the study area, a sex-ratio close to 0.94 was observed. The only significant difference from the 1:1 ratio was found in May 2006 (sex-ratio = 0.39). These results differ from those reported for the species in the western Mediterranean by Mangold-Wirz (1963), where the recorded sex ratio was significantly in favor of females, especially during the reproductive season. These variations in the sex ratio could be influenced by a combination of different factors, such as migrating behavior, associated with sexual maturation and spawning processes, as observed by Mangold-Wirz (1963) in the Mediterranean. The presence of a larger number of females in the larger length classes may be related to a different catchability between the two sexes and a fishing effort more aimed to females than to males due to the body shape dimorphism; females reach considerably larger adult sizes than males (Boyle, 1983).

The negative allometry in the length-weight relationships is in accordance with the results of other authors (Moriyasu, 1983; Lefkaditou & Papaconstantinou, 1995). During this study, an increase in length compared to weight was observed for immature specimens. Furthermore, the *b* value showed isometric growth in maturing and mature individuals, which indicates that in these phases both sexes gained more in weight than in length.

The length-somatic weight relationships indicated that this increase in the allometry coefficient in maturing and mature males is mainly related to the somatic growth, while in the same category of females the reproductive system had more intensive growth compared to somatic growth.

The smallest mature male and female analysed were respectively 5.8 and 6.5 cm DML, whereas the length at maturity was 6.88 cm DML for males and 8.80 cm DML for females. In comparison with the females, the maturity in

the males is more precocious, probably related in fact that the process of gonadic development in males requires less energy and so less nutritive reserve than in the female. Both males and females of *Eledone cirrhosa* in this study displayed lower DML_{50%} values than those obtained by Tursi et al. (1995) (7.7 cm in males, 8.9 cm in females) and Soro & Piccinetti Manfrin (1989) (9 cm and 11 cm in males and females respectively). These outcomes may be due to an adaptation to the specific environmental conditions. It seems that a series of environmental variables impact the reproductive biology behavior in this species, particularly temperature, which may influence the precocity of the sexual development. The relationship between high temperature and earlier gonad maturation was described in several studies (Forsythe, 2004) and it seems to be the main factor contributing to the sexual development process. In this respect, temperature conditions in waters deeper than 200 m in the northern and eastern sea of Tunisia is 13.5°C (Azouz, 1973), whereas at the same depth, the average of temperature is 13°C in the Aegean Sea and 11.5°C in the Adriatic Sea (Buljan & Zore-Armanda, 1971). The same observations were made about the musk octopus, *Eledone moschata* (Lamarck, 1798), from the gulf of Gabes (Ezzeddine-Najai, 1997) and the common cuttlefish, *Sepia officinalis*, from the gulf of Tunis (Ezzeddine-Najai, 1984), in which temperature plays an important role in the sexual maturity. In this regard, animals from warmer areas have precocious sexual maturity.

The GSI of both sexes was correlated noticeably with the mature fraction of the population. In the female, the decrease of the GI was manifested by the increase in gonad size, but in the male the increase of this index was related to the increase in number of spermatophores inside the Needham's sac. As a result of this analysis, it can be stated that the reproductive season in the northern and eastern region of Tunisia occurs from January to August, with a peak between April and August for males, and from April to August, with a peak between May and July for females. This conclusion is supported by the fact that females reach maturity later and at a wider range of sizes than males (Belcari et al., 1990). These results agree with those reported by Moriyasu (1988), Mangold et al. (1971) and Tursi & D'onghia (1992) respectively in the Gulf of Lion, the Catalan waters and The Ionian Sea. While, Tursi (1995) and Boyle & Knobloch (1983) showed an extensive spawning season of approximately four months

from July to September in the Aegean Sea and the eastern Atlantic. This would indicate that the spawning season and the reproductive peaks for *Eledone cirrhosa* are very flexible and closely linked to different environmental variables, as the life processes of cephalopods, including the initiation and duration of the reproductive season, are strongly affected by environmental factors, primarily temperature (Van Heukelem, 1979; Ezzedine-Najai, 1997).

The wide range of fecundity values, between 1859 and 8500 oocytes in mature females in the Tunisian northern and eastern areas, agree with those reported by Mangold-Wirz (1963) in the Catalan Sea. Boyle & Knobloch (1983) estimated a number of oocytes between 2000 and 54000 with a mean total fecundity of 11000 in the North Sea. This difference between populations of *Eledone cirrhosa* from different marine sectors is probably attributable to real differences between populations; the Scottish animals are of a much larger size (Boyle, 1983).

The correlation of the oocytes diameter with the dorsal mantle length was positive ($R^2 = 0.2989$), but it was clearly weaker than that obtained with the ovary weight ($R^2 = 0.5025$). Boyle (1983) also noted in the mature and maturing ovary, mean egg diameter is well correlated with total ovary weight. This result should be taken with precaution, because only the oocytes within the ovary were counted at the time of sampling, and we do not know the number of eggs that would have been already laid. In the same context, the weak correlation between fecundity and the female size may be attributed to the fact that some sampled females have already laid while others females in the same size class have not yet laid.

The diameters of oocytes in the mature ovary of *E. cirrhosa* from Tunisian waters measured between 1.5 and 7 mm. According to several authors, the way of life in the hatchings is dependent on the size of eggs. In *E. cirrhosa* of which the eggs are medium to large, the newly hatched animals have a very brief planktonic phase before adopting, within a few days, the benthic way of life (Mangold et al., 1971). The duration of planktonic life is very short to allow, in our opinion, a large spatial expansion for octopus. Each population is somehow constrained to adapt to its environment and thus the differences reported between *E. cirrhosa* population from Tunisian coasts and those of other marine areas would be given to environmental factors, especially temperature.

Moreover, the brevity of planktonic life in the newly hatched animals and the rapid adoption

to a benthic life may contribute to create populations genetically different in various marine areas. Molecular genetic studies would clarify this issue.

Despite the presence in the same ovary of oocytes at different sizes *E. cirrhosa* may be considered a total spawner with "synchronous ovulators" where the whole clutch of yolked oocytes ovulates at once and the eggs are shed over a short period of time, no longer than a month (Holden & Raitt, 1974).

In males, the maximum number of mature spermatophores was 121. This number was smaller than found by Mangold-Wirz (1963) in Mediterranean waters (150 spermatophores in May) just before the reproduction season. According to the low correlation ($r = 0.59$) obtained between the number of mature spermatophores and dorsal mantle length, the formation process of spermatophores is continuous in males, and individuals may breed more than once during the reproductive season. In fact, Boyle (1983) reported that copulation can occur many times over a significant proportion of the male life span and the males normally fertilize several females.

The present work is the first contribution to the knowledge of the reproductive biology of *Eledone cirrhosa* in the Tunisian northern and eastern waters. Reproductive parameters are determined and compared with those obtained in other marine areas. Further investigations involving the histological study of gonads and hydrographic factors would better elucidate the reproductive cycle of the species and environmental impact on the spawning process.

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DISTRIBUTION LIMITS OF THE GEODUCK CLAMS *PANOPEA GENEROSA* AND *P. GLOBOSA* ON THE PACIFIC COAST OF MEXICO

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ABSTRACT

Two species of geoduck clams, *Panopea*, are known from the Pacific coast of Mexico: *P. generosa*, also present in the temperate areas along the western coast of North America, and *P. globosa*, originally considered endemic to the Gulf of California. Often regarded as a temperate and a tropical species, their respective distributions were assumed to be clearly separate. However, commercial interests recently found populations in the temperate-tropical transition zone, thus raising doubts regarding their taxonomic identity and the distribution limits of each species. Discriminant function analysis was used to examine the morphological characteristics of the shells of individuals from nine locations to identify their species and to re-examine the distribution limits of *Panopea* spp. in this region. Results show that the tropical limit in the distribution of *P. generosa* is the western coast of the Baja California Peninsula, south to at least Punta Canoas (29°43'N). It was found that the distribution of *P. globosa* not only includes the Gulf of California but extends to at least Bahía Magdalena (24°38'N) on the western coast of the peninsula. This is the first report of *P. globosa* outside of the Gulf of California, thus extending its distribution to more temperate areas.

Key words: geoduck clam, Hiatellidae, *Panopea*, distribution, morphology.

INTRODUCTION

Geoduck clams (*Panopea* spp.: Hiatellidae) are well-known benthic species found in temperate waters in the Pacific off the coast of North America. *Panopea generosa* (Gould, 1850) sustains important commercial and recreational fisheries in Oregon, Washington and British Columbia (Orenzanz et al., 2004; Zang & Hang, 2006), (Vadopalas et al., 2010, recently reviewed the taxonomy of this species.) This has lead to a greater understanding of its biology and ecology due to ongoing management strategies in those areas (Bradbury et al., 2000a). *Panopea* species are long-lived, sedentary organisms that live buried in soft bottoms (Morsán & Ciocco, 2004; Gribben & Creese, 2005). As adults they are virtually free of natural predators, thus show low mortality rates (Breen & Shields, 1983; Harbo et al., 1983; Bradbury et al., 2000b). Their recruitment is also low (Goodwin & Shaul, 1984; Sloan & Robinson, 1984); therefore, their populations are relatively vulnerable to pressure of fisher-

ies. In addition, geoduck clams are sensitive to environmental conditions, including natural variations and the effects of anthropogenic changes (Valero et al., 2004).

Although not always regarded as fishery resources, geoduck clams are also present in tropical and subtropical areas of the Mexican Pacific, where export-oriented, controlled exploitations have been taking place in an increasing number of locations since 2002 (Aragón-Noriega et al., 2007; Calderón-Aguilera et al., 2010). The fishery started in a few areas in the upper Gulf of California, and also near El Rosario on the northwestern coast of the peninsula (30°4'N, 115°43'W). Whereas it became evident that catches included two species (*P. generosa* and *P. globosa*), the geographic distribution often considered for *P. generosa* (which includes the western coast of Baja California as its southern limit; see Goodwin & Pease, 1989), and the reports of *P. globosa* as endemic to the upper Gulf of California (Dall, 1898; Coan et al., 2000), lead to a general assumption that their respec-

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tive distributions were clearly separated, and therefore their identifications had been based principally on location: *P. generosa* for populations along the Pacific coast of the peninsula, and *P. globosa* for populations inside the Gulf of California (DOF, 2010).

In recent years, however, other populations have been located along the middle and southern areas of the Pacific coast of the peninsula, such as Punta Canoas (29°25'N) and Bahía Magdalena (24°38'N). Bahía Magdalena is regarded by some as the southern boundary in the distribution of *P. generosa* (e.g., after Goodwin & Pease, 1989, who considered this species to occur from Alaska to Baja California). At the same time, this area is considered the boundary between the Cortez Province (i.e., fauna from the Gulf of California) and the Californian Province (Brusca, 1980; Hastings, 2000), thus suggesting the possible existence of *P. globosa* in this area. Moreover, in their recent review Coan & Valentich-Scott (2012) pointed that records of *P. generosa* from Bahía Magdalena were based on misidentified *P. globosa*. This shows the need for a review of *a priori* ideas about the geographical distributions of both species in this region.

In a recent effort to clarify this issue, Rocha-Olivares et al. (2010) conducted a study comparing genetic and morphological differences of *P. generosa* from the northern Pacific coast of the peninsula and *P. globosa* from the upper Gulf of California; however, they did not consider specimens from the middle and southern portions of the western coast of the peninsula. In this work, we statistically analyzed the morphological characteristics of the shells of individuals from populations located along the Pacific coast of North America, to identify their species and to re-examine the distribution limits of *Panopea* spp. in the Mexican Pacific.

METHODS

Primary data for this analysis are morphological variables measured on 218 geoduck shell pairs collected from nine locations, from Vancouver, British Columbia, to the Gulf of California and including the Pacific coast of the Baja California Peninsula (Fig. 1). Shells were cleaned from soft tissue remains and dried, and then the right shell was weighed and placed on a surface with its internal side downward. To standardize measurements, shells were aligned considering the first com-

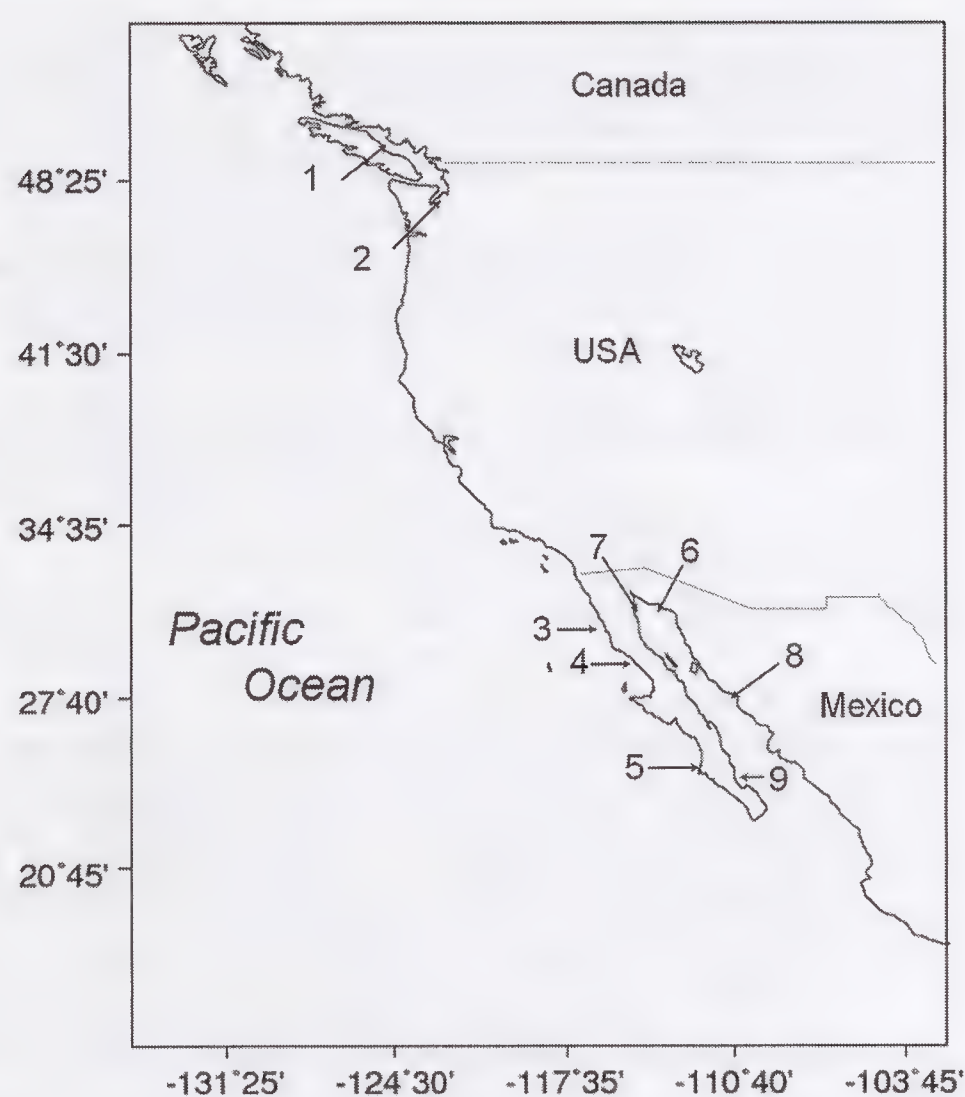


FIG. 1. Locations of the *Panopea* spp. shells considered in the analysis: (1) Vancouver, British Columbia; (2) Puget Sound, Washington; (3) San Quintín, Baja California; (4) Punta Canoas, Baja California; (5) Bahía Magdalena, Baja California Sur; (6) Puerto Peñasco, Sonora; (7) San Felipe, Baja California; (8) Guaymas, Sonora; (9) Bahía de la Paz, Baja California Sur.

marginal growth striae (proxy to 30–40 mm from umbo) as the horizontal reference. The shell length (SL), height (H), and the distance from the umbo to the posterior margin (UP) were measured using calipers (± 0.01 mm). The distance from the umbo to the ventral margin (UV) along the shell surface was determined using a flexible metric tape (± 0.01 mm). The distance from the umbo to the impression of the inner ligament layer in the nymph (UN) was measured in dorsal view on the internal surface of the shell with calipers. The width (WPS) and depth (DPS) of the pallial sinus were determined from digital images taken with an HP Scanjet 2200c scanner at 150 dpi, using the SigmaScan Pro 5.0 computer software. Finally, the single right shell was weighted (W) with a digital scale (± 1 g). A schematic representation of the morphological variables is presented in Figure 2, while their descriptive statistics of maxima, minima, mean and standard deviations are shown in Table 1.

In order to account for the effect of size in morphometric variables (i.e., to eliminate the variance component due to size differences

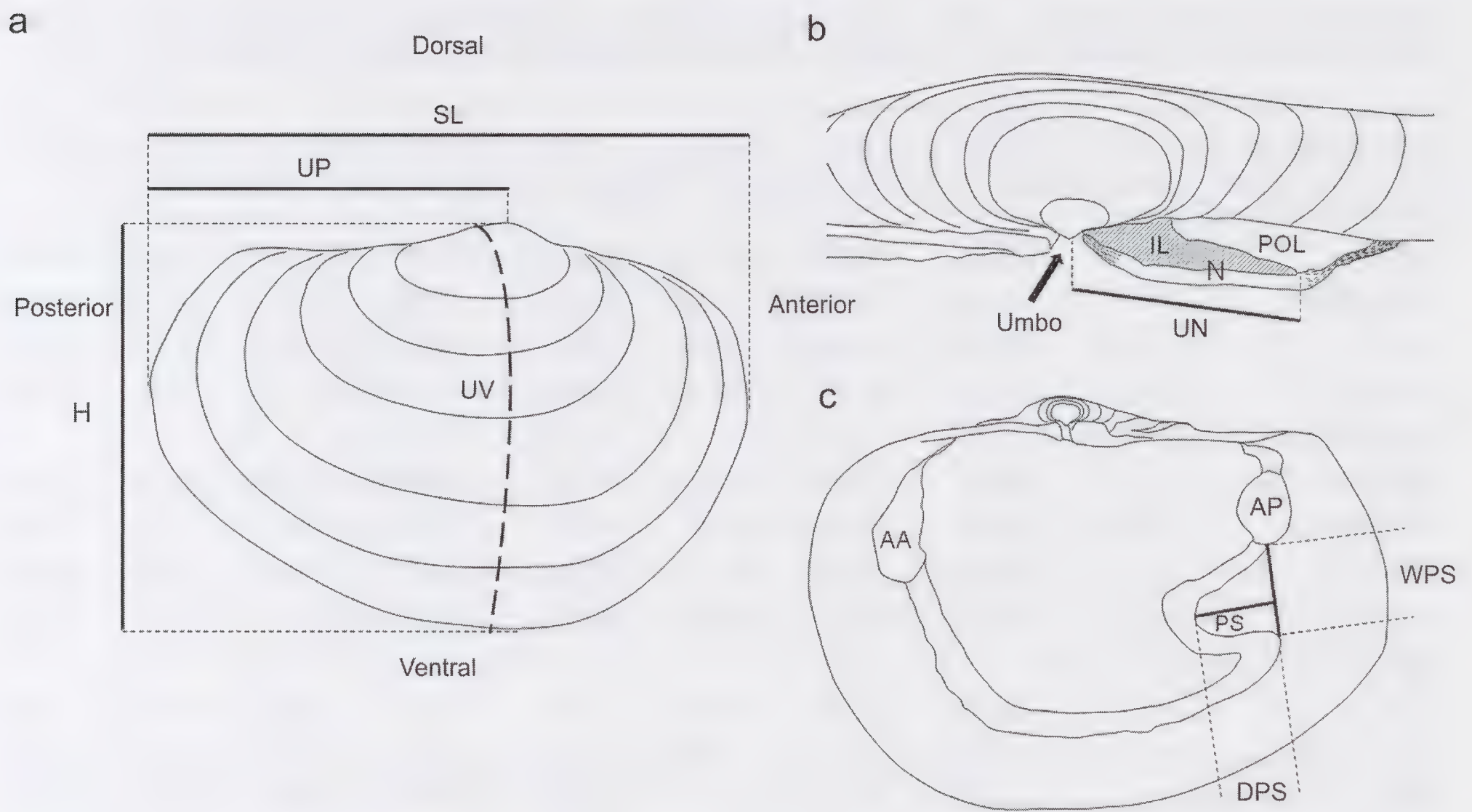


FIG. 2. Morphological variables considered in the analysis. Right lateral view (a): length (SL), height (H), distance from the umbo to the posterior margin (UP), and distance along the shell surface from the umbo to the ventral margin (UV). Dorsal view (b): distance from the umbo to the opposite end of the nymph (UN), nymph (N), inner ligament layer (IL) and posterior outer ligament layer (POL). Internal view (c): width (WPS) and depth (DPS) of pallial sinus, and adductor muscle scars, posterior (AP) and anterior (AA).

among individuals; see Humphries et al., 1981), we followed Rohlf & Bookstein (1987) and Bookstein (1991) in transforming the morphometric variables to proportions of linear measurements prior to their statistical analysis. For this, linear measurements of each individual (H, UP, UV, UN, WPS and DPS) were divided by the shell length (SL). Regarding weight, values were first transformed to logarithms and then divided by SL.

The transformed variables were analyzed using stepwise discriminant function analysis (DFA), a method that has proven effective at diagnosing molluscan species (Martínez et al., 2002). DFA is used to predict a categorical dependent variable by one or more independent variables, and is useful at determining whether a set of variables is effective in identifying category membership (Hill & Lewicki, 2007). DFA also provides linear combinations of the discriminating variables, called classification functions. One function per group can be generated from a sample of individuals for which group membership is known. These functions can be applied to new cases with measurements on the same set of variables

but unknown group membership; the highest value (score) obtained from the discriminant functions indicates that particular case belongs to the corresponding group.

We first applied DFA to determine which morphological (i.e., independent) variables are effective in identifying specimens as *P. generosa* or *P. globosa* (i.e., two categorical memberships). For this, we analyzed specimens from two populations that we considered *a priori* as representatives of each species: Punta Canoas ($n = 50$) for *P. generosa*, and Bahía Magdalena ($n = 50$) for *P. globosa*. For this, we followed the expert opinion of Valentich-Scott (Santa Barbara Museum of Natural History, California), who kindly agreed to examine and identify a few specimens from each location. This first DFA included all the morphological variables; then, a second DFA was made including only those variables that significantly differed between locations. Then, each specimen was statistically classified as a member of each group based on the Mahalanobis squared distances (D^2), and two classification functions were generated. To make sure that the variance component due to size differences was effectively removed by the

TABLE 1. Descriptive statistics of the morphological variables of 218 *Panopea* spp. shells from locations shown in Fig. 1. All measures are millimeters except for W (weight in grams).

Group	SL	H	UP	UN	UV	WPS	DPS	W
Vancouver (<i>n</i> = 2)								
Mean	145.50	95.00	89.00	23.24	127.00	20.37	15.89	129.50
Std. Dev.	10.61	7.07	15.56	0.40	2.83	1.13	5.17	9.19
Min	138.00	90.00	78.00	22.96	125.00	19.57	12.24	123.00
Max	153.00	100.00	100.00	23.52	129.00	21.17	19.55	136.00
Puget Sound (<i>n</i> = 5)								
Mean	125.38	77.63	72.88	12.52	99.75	23.86	9.32	37.00
Std. Dev.	23.28	8.01	15.00	1.31	11.41	3.54	1.23	7.79
Min	102.00	66.00	61.00	10.89	83.00	19.09	8.11	30.00
Max	157.00	84.00	94.00	14.05	107.00	27.11	10.81	46.00
San Quintín (<i>n</i> = 30)								
Mean	140.55	85.28	73.32	17.03	115.77	26.52	12.58	72.10
Std. Dev.	9.01	4.84	7.04	2.12	7.09	3.90	2.49	15.85
Min	118.00	72.00	62.00	12.24	100.00	19.68	8.94	43.00
Max	161.00	97.00	93.00	22.45	132.00	36.17	18.02	117.00
Punta Canoas (<i>n</i> = 50)								
Mean	117.14	72.18	69.38	11.83	94.10	19.77	11.11	31.52
Std. Dev.	18.47	9.99	11.63	2.72	13.03	3.05	2.25	15.19
Min	80	51	45	7	65	12.97	5.78	8
Max	149	92	96	18.06	118	27.34	16.00	64
Bahía Magdalena (<i>n</i> = 50)								
Mean	155.24	105.64	79.52	13.96	149.43	27.45	15.68	97.02
Std. Dev.	26.32	17.78	15.72	3.76	26.32	5.91	3.42	51.58
Min	90	55	35	6.85	76	16.22	9.91	10
Max	200	139	109	23.37	189	41.42	25.12	220
Pueto Peñasco (<i>n</i> = 25)								
Mean	158.88	108.64	96.00	15.05	154.88	31.26	19.60	89.00
Std. Dev.	13.47	6.42	9.23	1.99	9.06	3.10	3.71	18.06
Min	137.00	94.00	82.00	11.69	138.00	24.18	14.22	62.00
Max	198.00	120.00	125.00	19.42	174.00	36.52	27.01	126.00
San Felipe (<i>n</i> = 19)								
Mean	159.84	108.21	92.63	14.18	157.05	31.11	19.37	121.53
Std. Dev.	8.15	5.46	7.30	1.57	6.41	3.81	3.28	30.38
Min	150.00	94.00	82.00	11.70	143.00	20.25	14.39	69.00
Max	185.00	120.00	110.00	18.01	168.00	35.73	27.47	186.00
Guaymas (<i>n</i> = 7)								
Mean	99.00	70.71	58.07	9.03	102.14	19.51	11.12	21.86
Std. Dev.	19.17	13.68	12.72	2.06	21.61	2.99	1.94	15.56
Min	78.00	52.00	44.00	5.93	76.00	15.23	8.63	8.00
Max	135.00	95.00	82.50	11.71	143.00	25.20	13.59	54.00
Bahía de la Paz (<i>n</i> = 30)								
Mean	143.72	96.07	87.69	12.17	137.34	25.04	16.50	52.55
Std. Dev.	11.19	6.35	9.09	2.06	11.02	3.71	2.87	13.07
Min	112.00	74.00	66.00	9.13	100.00	16.22	10.41	20.00
Max	165.00	104.00	111.00	20.15	154.00	32.10	20.88	82.00

TABLE 2. Results of two stepwise DFA for specimens of *P. generosa* and *P. globosa*, showing the order of entry and the significance of the morphological variables (p-level). Variable abbreviations are as in Fig. 2.

	Step no.	Variable	Wilks' Lambda	Partial Lambda	F-remove	p-level	1-Tolerance (R ²)
First DFA	1	UV/SL*	0.3360	0.6206	56.25	0.0000	0.7769
	2	W/SL*	0.2456	0.8488	16.39	0.0001	0.6154
	3	DPS/SL	0.2164	0.9635	3.48	0.0652	0.1030
	4	UP/SL	0.2147	0.9712	2.73	0.1022	0.1930
	5	UN/SL	0.2145	0.9719	2.66	0.1061	0.2365
	6	WPS/SL	0.2140	0.9743	2.42	0.1230	0.3245
	7	H/SL	0.2091	0.9973	0.25	0.6200	0.7348
Second DFA	1	UV/SL*	0.9999	0.2452	298.63	0.0000	0.5202
	2	W/SL*	0.4039	0.6069	62.81	0.0000	0.5202

transformation of the morphological variables, we explored the linear regression between the highest discriminant value obtained for each specimen as a function of its size. Finally, the discriminant functions were applied to the specimens from other locations to statistically classify them and to determine the geographical distribution of each species.

RESULTS

The results of DFA for 50 specimens of *P. generosa* from Punta Canoas and 50 specimens of *P. globosa* from Bahía Magdalena are shown in Tables 2 and 3. Of the seven morphological variables included in this analysis, only two were statistically significant ($p < 0.01$) and therefore useful at discriminating between species: UV/SL (umbo to ventral margin distance over the shell length) and W/SL (natural logarithm of weight over the shell length). A second DFA for the same specimens but considering only these two significant variables yielded

TABLE 3. Results of two stepwise DFA for specimens of *P. generosa* and *P. globosa*, showing the percentages of correctly identified individuals.

Actual population (No. of specimens)	% Correct	<i>P.</i> <i>generosa</i>	<i>P.</i> <i>globosa</i>
<i>P. generosa</i>	98	49	1
<i>P. globosa</i>	100	0	50

classification functions that showed very high percentages of correct classifications: 98% for *P. generosa* and 100% for *P. globosa*. The coefficients of each morphological variable in the classification functions are provided in Table 4. Figure 3 presents the highest discriminant value obtained for each specimen

TABLE 4. Regression coefficients of the classification functions after the second DFA and their results for the analyzed populations.

Population	N	Proportion of specimens statistically classified as each species (%)	
		<i>P. generosa</i>	<i>P. globosa</i>
Vancouver	2	100	0
Puget Sound	5	100	0
San Quintín	30	100	0
Punta Canoas	50	98	2
Bahía Magdalena	50	0	100
Puerto Peñasco	25	0	100
San Felipe	19	10.5	89.5
Guaymas	7	0	100
Bahía de La Paz	30	0	100

Variable	Coefficients	
UV/SL	52.52	129.76
W/SL	6607.90	4696.10
Constant	-115.50	-129.71

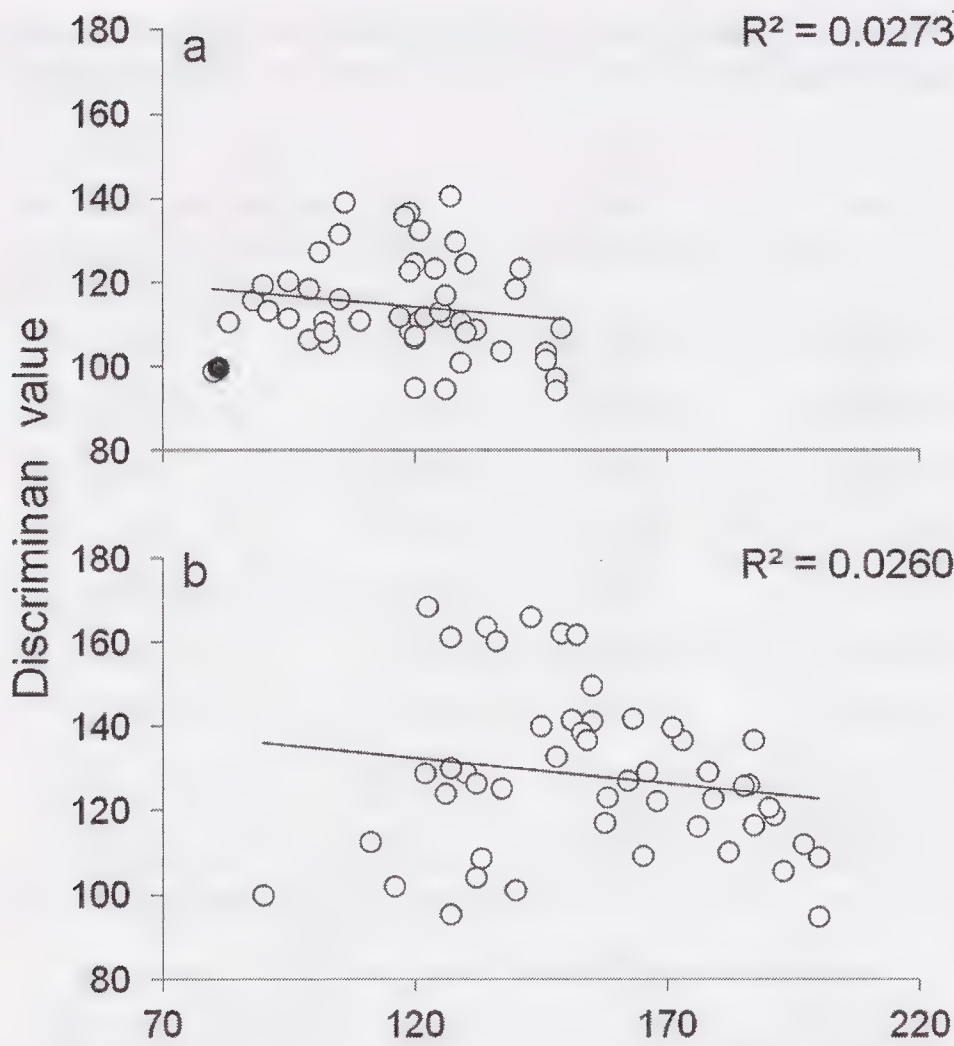


FIG. 3. Shell length plotted against the highest discriminate value obtained for all specimens, identified as (a) *P. generosa* (one individual that resulted in misidentification is marked as solid circle ●) and (b) *P. globosa*.

plotted against the corresponding size. The regression line for each species show no evidence of a linear trend, thus indicating that any significant effect of size was effectively removed by the transformation of the morphological variables. After applying the clas-

sification functions to the samples from other locations, all specimens from San Quintín (n = 30), as well as those from Vancouver (n = 2) and Puget Sound (n = 5) resulted statistically classified as *P. generosa*. For locations in the Gulf of California, all specimens from Puerto Peñasco (n = 25), Guaymas (n = 7), Bahía de La Paz (n = 30) and most specimens from San Felipe (17 of 19) were classified as *P. globosa* (Table 4).

DISCUSSION

Since the early work of Dall (1898), there have been changes regarding the recognized distribution of both *Panopea* species along the Pacific coast of North America. In particular, the tropical limit of *P. generosa* was first considered by Dall (1898) to be “south to the Gulf of California”. Coan et al. (2000) placed its limit at Newport, California, and Moore (2001) included the northern Gulf of California as part of the species distribution. For *P. globosa*, since its original description as a subspecies of *P. generosa* by Dall (1898), followed by Keen (1971), Coan et al. (2000), Hendrickx et al. (2005), and Aragón Noriega et al. (2007), its distribution has been regarded as the northern to central Gulf of California.

From our results, we propose that the tropical limit in the distribution of *P. generosa* is the western coast of the Baja California Peninsula, south to at least Punta Canoas (29°43’N).

TABLE 5. Comparison of some morphological characteristics of *P. generosa* and *P. globosa*. See also Figs. 5–9.

<i>P. generosa</i>		<i>P. globosa</i>
Shell shape	Subquadrate, relatively flat	Rounded (subquadrate in small specimens), anterior margin lobe, more inflated
Anterior margin	Rounded	Wide rounded
Posterior margin	Truncate, narrowly gaping	Rounded, slightly, acute
Dorsal margin	Parallel to major axis	Not parallel to major axis
Ventral margin	Nearly straight, parallel to dorsal margin	Rounded (parallel to major axis in small specimens)
Umbo (beaks)	Nearly medial	Slightly shifted toward the posterior margin
Nymph	Long	Short
Ligament	Wide, V-shaped (internal view)	Short, U-shaped (internal view)
Pallial sinus	Shallow	Deep
Pallial line	Thick	Thin

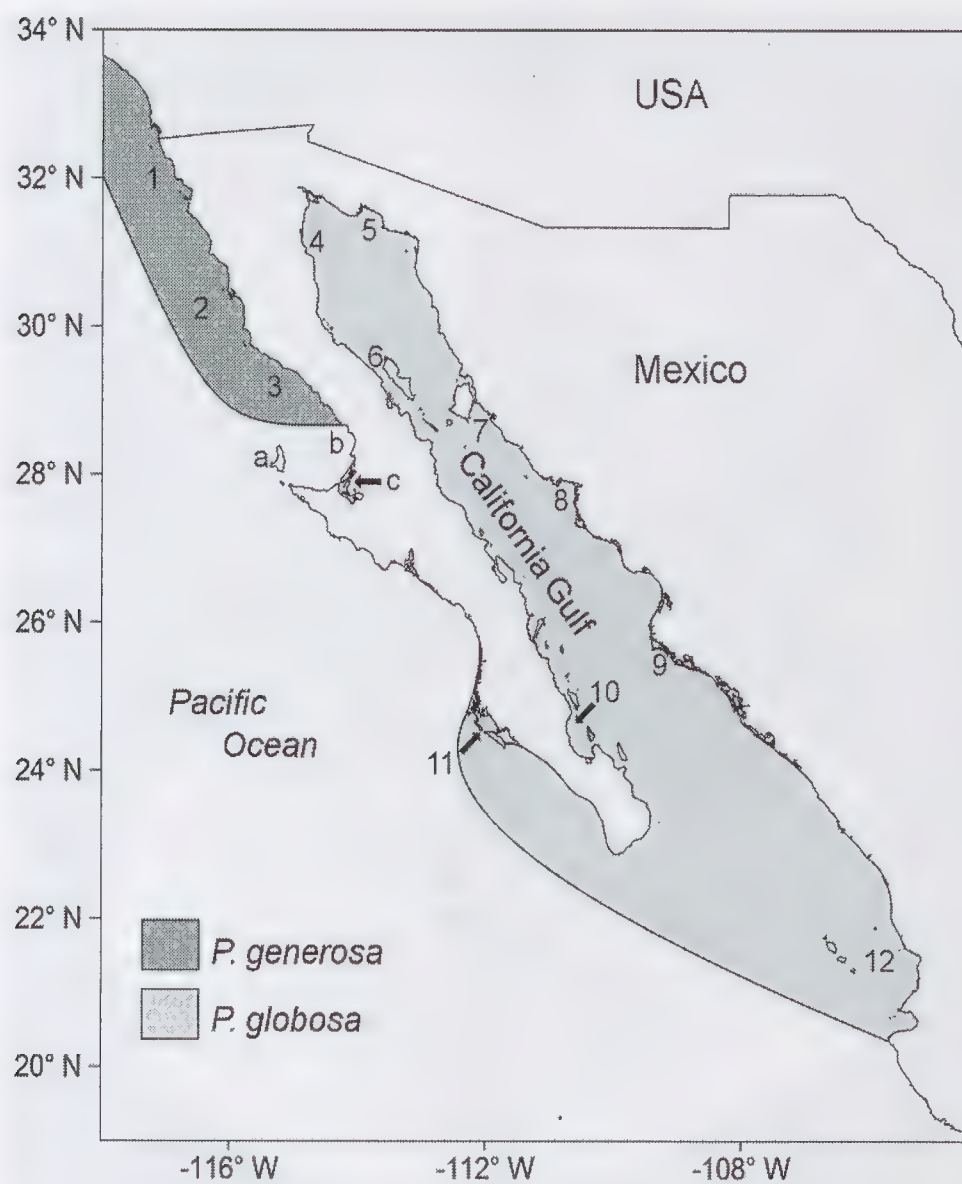


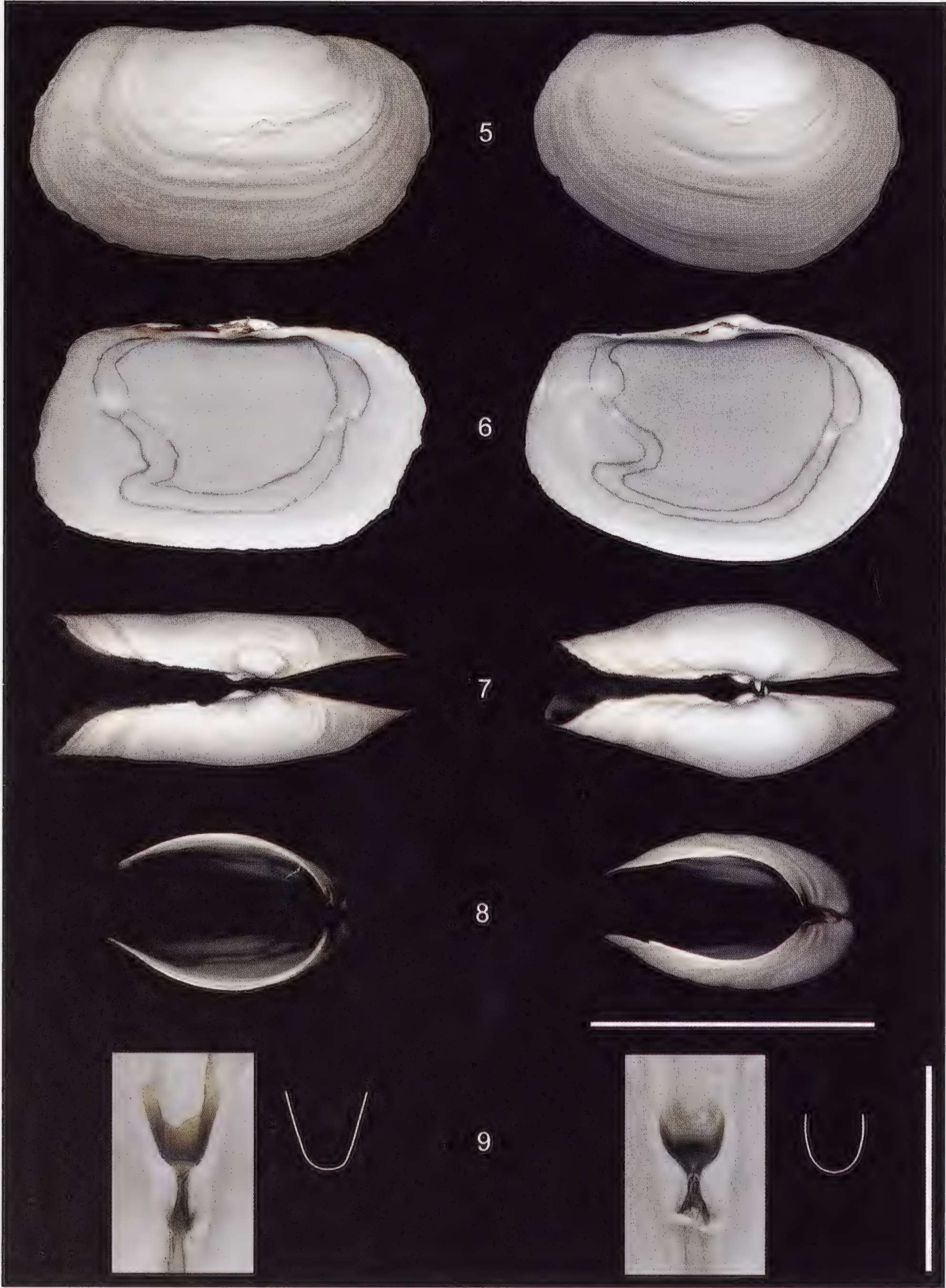
FIG. 4. Distribution of *Panopea* spp. in western Mexico. Confirmed locations for *P. generosa*: (1) Salsipuedes (SBMNH 83184); (2) San Quintín (our results) and Playa Santa María (SBMNH 123406); (3) Punta Canoas (our results). Confirmed locations for *P. globosa*: (4) San Felipe (our results and SBMNH 125392, 16831, 149354, 30300, 42235, 149353, 149355 and 83183); (5) Puerto Peñasco (our results); (6) Bahía de Los Angeles (INP-CONAPESCA, 2010); (7) Bahía de Kino (SBMNH 30325); (8) Guaymas (our results and SBMNH 149356, 347804, 351970); (9) Bahía de Topolobampo (SBMNH 30299); (10) Bahía de La Paz (our results); (11) Bahía Magdalena (our results); (12) Playa Novillero (SBMNH 135157). Locations with unconfirmed reports of geoduck clams: (a) Isla Cedros; (b) San José de las Palomas; (c) Guerrero Negro.

Regarding the two specimens from San Felipe that were statistically classified as *P. generosa*, they were probably misclassified, since most specimens resulted *P. globosa*, and it is reasonable to assume that the sample came from a single population. This is consistent with the idea that earlier records of *P. generosa* from Bahía Magdalena were based on misidentified *P. globosa* (Coan & Valentich-Scott, 2012). In addition, in a recent contribution Leyva-Valencia et al. (in press) showed that fossil *Panopea generosa* var. *taeniata* Dall, 1918, from Bahía Magdalena is actually a morphotype of *P. globosa*.

For *P. globosa*, its distribution reaches the tropical-temperate transition zone on the western coast of the peninsula, at least to Bahía Magdalena (24°38'N). Again, the one specimen from Punta Canoas that was statistically classified as *P. globosa* (only one of 50) was probably misclassified. The proposed limits are consistent with reports on the species and locations of geoduck clams in the Pacific coast of Mexico, including records of specimens deposited in the Santa Barbara Museum of Natural History (SBMNH). Taking all of this information into account, a revised distribution of each species in Mexico is presented in Figure 4. For *P. globosa*, it is evident that its distribution is not restricted to the Gulf of California. Not only is it found along the Pacific coast of the peninsula, but also it has been found as far south as Playa Novillero, Nayarit (22°22'N). For *P. generosa*, current evidence places its tropical limit at Punta Canoas (29°43'N), but it is likely that its distribution extends further south, as suggested by unconfirmed reports by fishermen of geoduck clams in San José de las Palomas (28°42'N), Isla de Cedros (28°11'N) and Guerrero Negro (27°58'N).

Regarding the species identification, a comparison of some distinctive characteristics is presented in Figures 5–9 and Table 5. Whereas the classification functions provided here proved useful, some additional notes might also be helpful at distinguishing between both species in practice. Overall, shells of *P. globosa* are heavier, shorter and more inflated than those of *P. generosa*. Also, the length of the nymph in *P. globosa* is shorter. In particular, we noted that the shape of the nymph ligament, U-shaped in *P. globosa* and V-shaped in *P. generosa*, is a distinctive character that may prove very useful for identification purposes.

Summarizing, we believe that our results provide a clearer picture on the limits of the distribution of these two *Panopea* species. However, because the continued commercial interest on this resource could lead to the detection of previously unknown populations along the Pacific coast of Mexico, these limits might need further review in the near future. In particular, and although no specimens of either species have been located between Punta Canoas and Bahía Magdalena, it should be noted that virtually no attention has been paid regarding areas deeper than those accessible to commercial divers. Therefore, at this point, the possibility of overlapping distributions cannot be completely ruled out.



FIGS. 5–9. Comparisons of shells of *P. generosa* (left) and *P. globosa* (right). FIG. 5: Lateral exterior view; FIG. 6: Lateral interior view; FIG. 7: Dorsal view; FIG. 8: Posterior view (horizontal scale bar = 100 mm). FIG. 9: Internal view of the articulated nymphs and ligaments in V-shaped and U-shaped (vertical scale bar = 40 mm). See also Table 5.

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MICROHABITAT REQUIREMENTS OF FIVE RARE VERTIGINID SPECIES (GASTROPODA, PULMONATA: VERTIGINIDAE) IN WETLANDS OF WESTERN POLAND

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ABSTRACT

Microhabitat requirements of five vertiginid species – *Vertigo angustior*, *V. moulinsiana*, *V. pygmaea*, *V. substriata* and *Columella edentula* – were studied in two wetland areas of western Poland; two of the species, *V. angustior* and *V. moulinsiana*, are respectively listed as “near threatened” and “vulnerable” according to the IUCN red list. Data on the species and their habitats were collected biweekly during spring and summer months in 2008 and 2009 along four permanent transects at each site. Factors influencing the distribution of vertiginids were examined using Canonical Correspondence Analysis and Canonical Varieties Analysis. A variance partitioning approach was used to determine the influence of groups of factors.

The small-scale distribution of the vertiginids was strongly influenced by specific environmental conditions. Both abiotic microhabitat characteristics and vegetation structure significantly influenced the composition of the studied malacocenoses. The most important factors were: litter moisture, shading, ground water level and the abundance of the following plants species: *Carex acutiformis*, *Thelypteris palustris*, *Lysimachia vulgaris* and *Urtica dioica*. Within the habitats covered by the study, abiotic factors were more important than vegetation composition, although the influence of two groups of variables was partially overlapping.

Key words: microhabitat preferences, fens, marshes, CCA, variance partitioning.

INTRODUCTION

Most of the species of the land snail family Vertiginidae are found in well-preserved wetland habitats within temperate climate zones, such as fens and reed-sedge marshes. As a consequence, ranges and abundance in populations of these species are in decline, due to habitat degradation and destruction as a result of human activities and climate change (Pokryszko, 2003). Detailed knowledge of microhabitat requirements is therefore essential for the efficient protection of vertiginid species and their habitats (Speight et al., 2003).

Among the Vertiginidae, two lowland European species: *Vertigo angustior* Jeffreys, 1830, and *V. moulinsiana* (Dupuy, 1849) are regarded as endangered across Europe and listed on the IUCN red list (IUCN, 2012) and in Annex II of the European Union’s Habitats Directive. Although their general habitat preferences are known (e.g., Boycott, 1934; Juříčková &

Kučera, 2005; Juříčková & Ložek, 2008; Vavrová et al., 2009), knowledge of their microhabitat requirements is geographically limited. For *V. angustior*, Cameron (2003) provided a detailed microhabitat analysis at one site in Great Britain, and Cameron et al. (2003) summarized further studies in Great Britain. More detailed data can be found in recent studies by Moorkens & Killeen (2011) and Killeen & Moorkens (2011). This species requires a permanently damp but not inundated substratum, with thin vegetation cover supplying abundant litter (Cameron et al., 2003) with such conditions being stable across several years (Norris & Colville, 1974). Thus, *V. angustior* appears to be associated with a particular combination of environmental conditions rather than specific plant communities.

According to Killeen (2003), *V. moulinsiana*, the other endangered species, occupies permanently wet, open habitats, and its abundance strongly depends on ground wa-

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ter level. Tattersfield & McInnes (2003) also investigated the hydrology of *V. moulinsiana* habitats around Great Britain. In the case of this species, local hydrology seems to be the major factor determining its small-scale distribution, although most of the records suggest that *V. moulinsiana* is associated with reed swamps. However, there are only a few studies that have analysed these relationships basing on direct measurements and statistical analyses (Martin & Sommer, 2004; Horsák et al., 2007).

Most of the data on microhabitat preferences for the two endangered vertiginid species comes from localities on the western limits of their ranges (mainly Great Britain). There is a lack of such data from central and eastern Europe, where these species occupy slightly different habitats (Pouličková et al., 2005; Juříčková & Ložek, 2008). The aim of the present study is to fill this gap using a detailed analysis of the microhabitat preferences of *V. moulinsiana*, *V. angustior* and three accompanying vertiginid species: *V. pygmaea* (Draparnaud, 1801), *V. substriata* (Jeffreys, 1833) and *Columella edentula* (Draparnaud, 1805) from two lowland river valley localities in western Poland.

MATERIAL AND METHODS

Study Area and Sampling

The study was conducted in two localities within the valleys of two small lowland rivers: Pliszka and Ilanka. These tributaries of the river Odra cross the Lubuskie Lakeland in western Poland. The lengths of rivers do not exceed 65 kilometres (Wołejko & Stańko, 1998). The bottom of the valleys are locally covered by damp meadows and pastures, surrounded by oak and pine forests. The composition of the present plant species indicates that they had been calcareous fens in the past (Stańko & Wołejko, 2001; Wołejko et al., 2001).

At the site on the Pliszka River (52°14'46.5"N, 15°10'11.1"E; ca. 0.20 ha in area) the level of ground water varied from -20 to +5 cm above the ground surface during the period of our study. The majority of the area is open and was lightly pastured in the past (personal communication from the owner). Approximately 35% of the locality is permanently marshy. The average litter moisture in the microhabitats sampled during two seasons was 74.9% ($\sigma = 15.7$).

Diversity of the microhabitats and plant species composition is high and the moss layer is well formed. Such plants as *Carex acutiformis*, *Carex paniculata* and *Thelypteris palustris* are abundant. The locality is protected as a part of European Natura2000 network area (Code: PLH 080011).

The site in the Ilanka River valley (52°20'09.7"N, 15°03'07.3"E; ca. 0.15 ha in area) is notably homogeneous and mostly covered by *Carex acutiformis*. The habitat is relatively eutrophicated (indicated by the presence of nettle, *Urtica dioica*), moderately humid and was never flooded in the last ten years. Ground water level varied from -20 cm to -5 cm during the period of our study and the average litter moisture was 78.5% ($\sigma = 17.8$).

The habitat is mostly open: only the northern section is partly shaded by alder. About 60 years ago, the sedge marsh was extensively used as a pasture. The site is a part of a Natura2000 area (PLH080009).

Both localities were sampled biweekly during the spring and summer months in 2008 (nine sampling events in Pliszka, ten in Ilanka) and 2009 (seven – Pliszka; seven – Ilanka) (five samplings were later excluded from the statistical analyses – see the Statistical Analyses section). At each locality, four parallel transects were laid out. Along each of the transects four samples of litter were collected at 10 m intervals using a 0.25 x 0.25 cm Økland frame (0.0625 m² in area) (Økland, 1929).

Sharland's methodology was used for sampling (Cameron, 2003): we collected all loose litter and dead vegetation, cut out the soil and root-mat to a depth of 5 cm and cut and collected all vegetation to the ground level. In addition, the data for the moisture of the litter, shading and percentage cover of prevailing plant species were recorded for each quadrat.

The data on litter moisture were obtained using a Nortene 5070141 hygrometer. Shading was assessed in a three-degree scale, where 1 described plots void of any shading, 2 – temporarily shaded by trees or bushes and 3 – squares permanently shaded. Percentage cover of the most common plant species on each 25 x 25 cm quadrat was determined each time before the sample was taken. For each sampling plot along the transects, range of ground water level during the study was measured using a piezometer and characterised by 5-degree scale: (1): -15 cm to -20 cm; (2): -10 cm to -15 cm, (3): -5 cm to -10 cm;

(4): 0 to -5 cm; (5): water above the ground surface.

In the laboratory, litter and soil were dried and divided into two fractions using a 0.5 mm sieve. Particles passing through the sieve were checked manually for presence of snails under a stereo microscope. The retained fraction and cut plants were carefully examined for snails using a magnifying glass. Only living individuals were considered for statistical analyses.

Statistical Analyses

To test the influence of microhabitat parameters on the structure of vertiginid assemblages, a series of Canonical Correspondence Analyses (CCA) were conducted (Braak & Šmilauer, 2002). We used the following environmental factors as explanatory variables: litter moisture, shading, ground water level and percentage cover of four most common plants of the study sites (*Carex acutiformis*, *Urtica dioica*, *Thelypteris palustris*, *Lysimachia vulgaris*).

First, a general CCA model was created to check for the relationships that were common for both localities. All data on environmental factors and snail densities for both sites were introduced into this model and grouped using covariables separating the blocks of data from particular localities and sampling seasons. Next, in order to check if there were any differences in significance of particular environmental factors between the localities, two other CCA models were used, analysing the data from each locality separately.

Forward selection of environmental variables was applied while developing the models. To determine the statistical significance of explanatory variables as well as the significance of the models created, a Monte Carlo permutation test set for 5,000 permutations was applied (Jongman et al., 1995; Braak, 1996). To minimize the influence of the data autocorrelation, the permutations were restricted for split-plot design, with blocks of data from particular seasons and localities regarded as independent whole-plots constituting four time series. The data from each sampling event were regarded as split-plots consisting of sampling plots arranged in 4 x 4 grid (four transects x four samples), nested within the whole-plots (Lepš & Šmilauer, 2003). The weight of species with low occurrence in the data was decreased in the analyses using the downweighting algorithm of the program (Braak & Šmilauer, 2002).

Vegetation structure of wetlands strongly depends on environmental variables such as moisture or insolation. On the other hand, those variables per se depend on the plant cover. To distinguish between the influence of plant composition and local abiotic variables on the vertiginid communities studied, a variation partitioning approach was used (Borcard et al., 1992). The environmental variables used in the previous analyses were grouped in two sets, first comprising the abiotic factors and the second including the vegetation data. Densities of vertiginid populations recorded during each sampling event were included as dependent variables. Next, variation partitioning models were created to separate the contribution of factors from both groups in explaining the structure of vertiginid communities. Following the approach by Borcard et al. (1992), five separate CCA analyses were performed, each of them comprising data on abiotic and biotic factors separately or accompanied with variables from the second group as covariables. Models were developed and tested in the same way as the general CCA model described above. The results were compared with respect to the variance in species data explained by each model.

The influence of environmental factors on the occurrence of individual snail species was analysed by series of CVA analyses with forward selection and Monte Carlo test (Lepš & Šmilauer, 2003). We followed the same assumptions regarding the model structure and permutation design as in the case of the general CCA test.

All the raw data used for the analyses is available in an Excel file from the senior author upon request and from the Internet site: www.zzo.amu.edu.pl/datasets. In some cases, when individual records were missing, they were replaced by means for corresponding sampling event (Jongman et al., 1995) and marked in the data file (as 1 in the "Missing" column). In the case of five sampling events, there was too much missing data, and whole blocks representing such events were excluded from the analyses (marked as "Delete" in the database). Thus, the final analyses were conducted on the data for 13 sampling events on Pliszka locality (seven in 2008 and six in 2009) and 15 for Ilanka locality (eight in 2008 and seven in 2009).

In all the statistical analyses, we considered $P = 0.05$ as the minimum level when determining significance. All the analyses were performed using CANOCO for Windows 4.5 software package (Braak & Šmilauer, 2002).

TABLE 1. Number of individuals, mean abundance and constancy of occurrence of vertiginid species in two wetlands in western Poland.

Locality	Species	No. of individuals	Mean abundance [individuals per m ²]	Constancy across samples [%]	Coefficient of variation in species abundance [%]
Ilanka	<i>V. angustior</i>	5722	370.7	82.5	1.83
	<i>V. moulinsiana</i>	106	6.9	18.2	3.24
	<i>V. substriata</i>	0	0.0	0.0	—
	<i>V. pygmaea</i>	143	9.2	23.4	2.89
	<i>C. edentula</i>	512	32.8	65.2	1.33
Pliszka	<i>V. angustior</i>	852	64.6	67.3	1.36
	<i>V. moulinsiana</i>	196	14.5	13.4	3.90
	<i>V. substriata</i>	376	28.0	58.2	1.50
	<i>V. pygmaea</i>	0	0.0	0.0	—
	<i>C. edentula</i>	10	0.8	2.8	6.14

RESULTS

Four of the studied species were recorded from the Ilanka River valley locality and four were recorded from Pliszka River valley locality. Table 1 has the basic parameters of populations in each area.

The most common species in the Ilanka locality was *Vertigo angustior*, occurring in 82.5% of the samples. Nevertheless, *Columella edentula* was the most uniformly distributed species, as shown by the low coefficient of variation in its densities across samples (CV = 1.33%). The least frequent species in this locality was *Vertigo moulinsiana*, occurring in 18.2% of samples. This species was also the most aggregated one as shown by the high coefficient of variation (CV = 3.24%).

Similarly, the most frequent species in the Pliszka locality was *Vertigo angustior*, recorded in 67.3% of samples. This species was also the most uniformly distributed (CV = 1.36%). Contrary to the situation observed in Ilanka, *Columella edentula* was the most aggregated species in this locality (CV = 6.14%).

The small-scale distribution of the studied vertiginid species was dependent on both abiotic characteristics of habitat and plant species composition on the sampling site. Results of forward selection in the general CCA model (Fig. 1) showed that among abiotic factors the most significant were litter moisture, shading and ground water level (Table 2 has the detailed statistics). The plant species that

significantly added to the model were *U. dioica* and *T. palustris*. The model was significant at $p < 0.0002$; $F = 7.983$.

Results of forward selection in the CCA model for Pliszka locality are shown in Table 3. The most important abiotic factors shaping snail communities were found to be shading and litter moisture. Among the variables describing vegetation, only percentage cover of *L. vulgaris* significantly improved the model according to the results of forward selection. The model was significant at $p < 0.0002$; $F = 4.882$. In the CCA model for Ilanka locality ($p < 0.0002$; $F = 10.079$), all the factors were found to be significant, with litter moisture and shading as the most important ones (Table 4).

According to the general and Pliszka model, *V. angustior* was the most characteristic for assemblages occurring in plots void of shading, with moderate ground water level and *T. palustris* abundance (Figs. 1, 2). In the Ilanka locality, the species co-created associations linked to moderate litter moisture as well as low abundance of *U. dioica* and *T. palustris* (Fig. 3). According to the results of CVA (Table 5), factors significantly favoured by *V. angustior* were high litter moisture and ground water level. Moreover, this species occurred in places where *T. palustris* was more abundant and shading was the lowest.

According to the general CCA model, *V. moulinsiana* occurred mainly in molluscan communities formed on highly shaded plots with abundant *L. vulgaris* (Fig. 1). On the Pliszka locality the

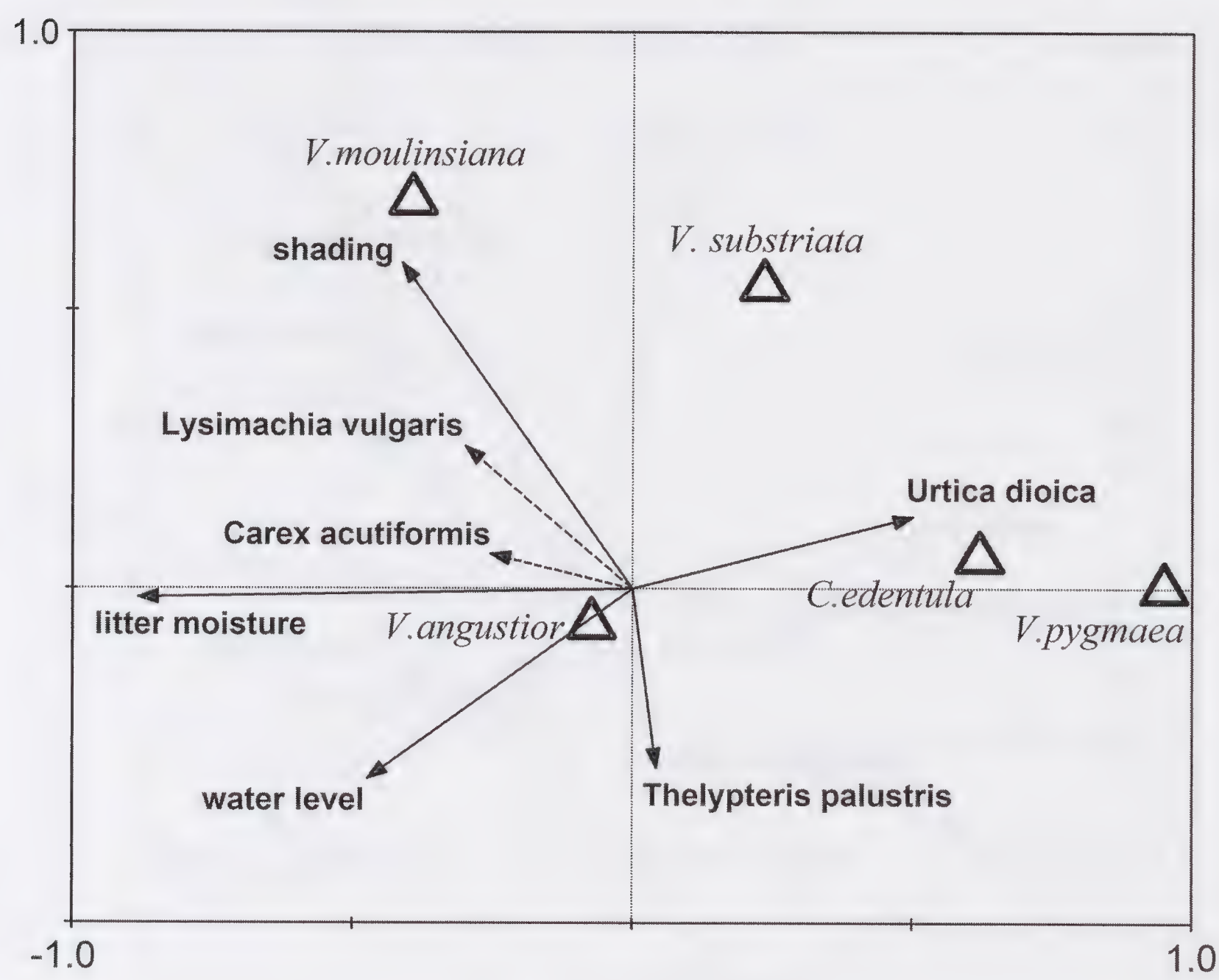


FIG. 1. Canonical Correspondence Analysis diagram showing the relationships between abiotic microhabitat factors, share abundance of plant species and densities of vertiginids recorded in two marshes during the study (triangles). Factors statistically significant are marked by solid arrow lines; statistically insignificant variables: dashed arrow lines. Model significance (Monte Carlo permutation test): $p < 0.0002$; $F = 7.983$.

malacocenoses dominated by *V. moulinsiana* formed mainly in plots with *Carex acutiformis* and *Lysimachia vulgaris*. The moderate-high litter moisture and shading were preferred (Fig.

2). In the Ilanka site, the species occurred in associations linked to shaded plots with higher ground water level and litter moisture, covered by *L. vulgaris* and *T. palustris* (Fig. 3). According to

TABLE 2. Results of the general Canonical Correspondence Analysis testing for the environmental factors influencing the distribution of five vertiginid species in both marshes studied.

Variable	LambdaA	F	P
litter moisture	0.13	23.62	< 0.001
shading	0.05	8.56	< 0.001
water level	0.05	9.62	< 0.001
<i>Urtica dioica</i>	0.02	4.17	0.004
<i>Thelypteris palustris</i>	0.02	3.66	0.013
<i>Lysimachia vulgaris</i>	0.02	3.20	0.071
<i>Carex acutiformis</i>	0.00	1.16	0.364

TABLE 3. Results of the Canonical Correspondence Analysis testing for the environmental factors influencing the distribution of five vertiginid species in the Pliszka marsh.

Variable	LambdaA	F	P
shading	0.18	18.22	< 0.001
litter moisture	0.09	8.78	0.001
<i>Lysimachia vulgaris</i>	0.03	3.16	0.018
<i>Urtica dioica</i>	0.01	1.29	0.324
<i>Thelypteris palustris</i>	0.01	1.09	0.123
<i>Carex acutiformis</i>	0.01	0.54	0.579
water level	0.00	0.25	0.885

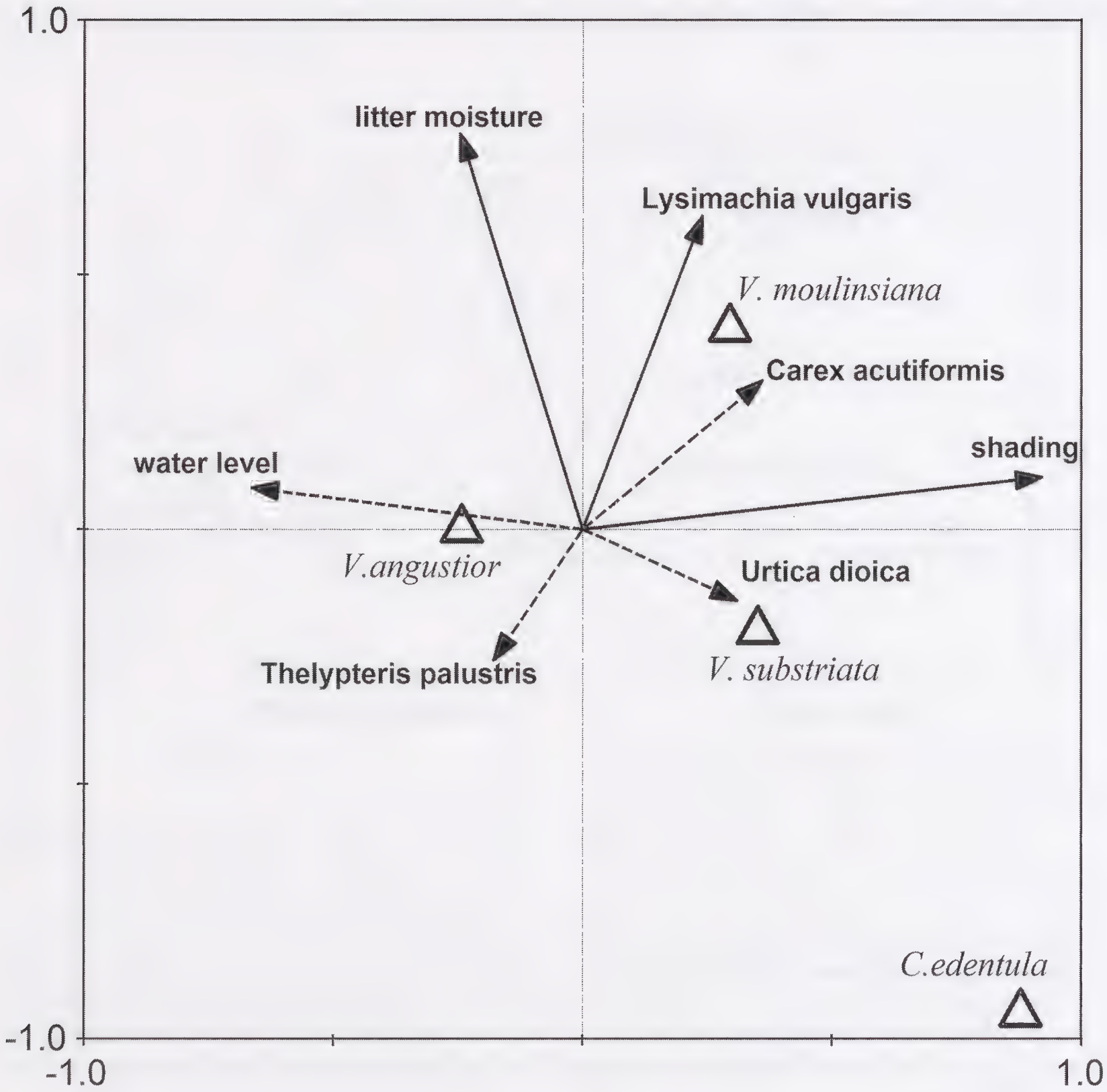


FIG. 2. Canonical Correspondence Analysis diagram showing the relationships between abiotic microhabitat factors, share abundance of plant species and densities of vertiginids recorded in the Pliszka marsh (triangles). Factors statistically significant are marked by solid arrow lines; statistically insignificant variables: dashed arrow lines. Model significance (Monte Carlo permutation test): $p < 0.0002$; $F = 4.882$.

TABLE 4. Results of the Canonical Correspondence Analysis testing for the environmental factors influencing the distribution of five vertiginid species in Ilanka marsh studied.

Variable	LambdaA	F	P
litter moisture	0.13	20.60	< 0.001
shading	0.11	18.37	< 0.001
<i>Thelypteris palustris</i>	0.06	9.56	0.012
<i>Carex acutiformis</i>	0.03	5.59	0.006
<i>Urtica dioica</i>	0.03	5.40	0.004
water level	0.03	4.31	0.005
<i>Lysimachia vulgaris</i>	0.02	3.26	0.043

the CVA analysis, factors significantly influencing occurrence of the species were litter moisture, *L. vulgaris* cover (positively), ground water level and *T. palustris* cover (negatively). Communities with abundant *Vertigo substriata* were found in shaded plots characterized by a relatively high abundance of *Urtica dioica* and low litter moisture as well as ground water level (Figs. 1, 2). Results of CVA showed a positive affinity for this species to shaded plots as well as *T. palustris* and *C. acutiformis* cover and negative to ground water level (Table 5). *Columella edentula* and *V. pygmaea* formed a community linked with *U. dioica* presence. The two snail species occurred within the least humid plots (Figs. 1, 3). CVA analysis showed

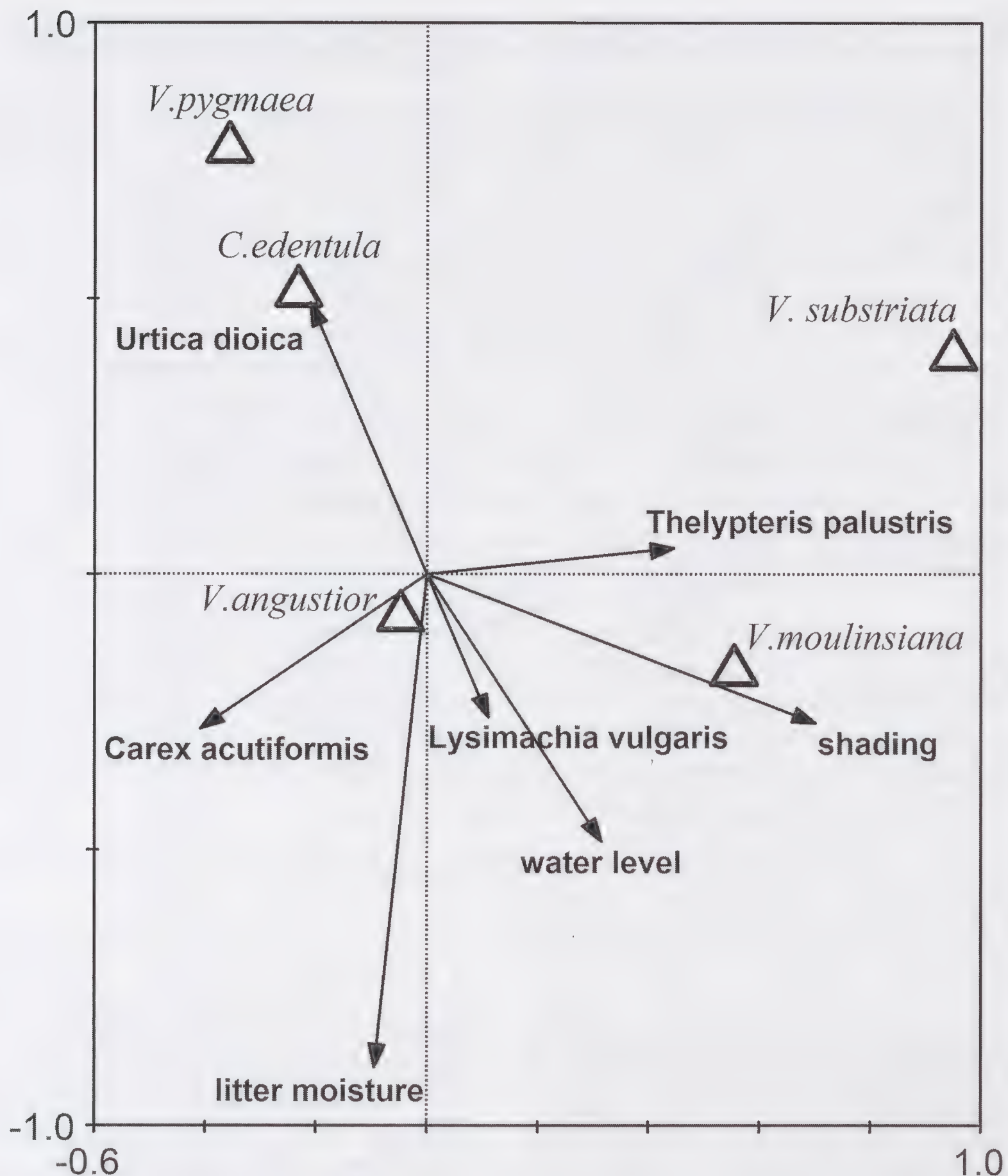


FIG. 3. Canonical Correspondence Analysis diagram showing the relationships between abiotic microhabitat factors, share abundance of plant species and densities of vertiginids recorded in the Ilanka marsh (triangles). Factors statistically significant are marked by solid arrow lines. Model significance (Monte Carlo permutation test): $p < 0.0002$; $F = 10.079$.

that *C. edentula* preferred places with high share of *U. dioica* as sole factor significantly influencing the occurrence of species. *Vertigo pygmaea* also had a preference for plots covered by *U. dioica* but avoided the waterlogged and shaded areas (Table 5).

Results of the variance partitioning models (Table 6) show that the influence of abiotic factors was more important for the five snail species than the plant composition. The majority of the variance explained by vegetation related factors was common to that explained by the abiotic factors.

DISCUSSION

Factors Shaping Molluscan Communities

Habitat requirements of *Vertigo moulinsiana*, *V. angustior*, *V. substriata*, *V. pygmaea* and *Columella edentula* are well known, nevertheless knowledge about microhabitat preferences across the wide geographical ranges of the species is scarce.

Most of the authors indicate abiotic factors as the most important in shaping molluscan species richness and distribution (Boycott, 1934;

TABLE 5. Results of Canonical Varieties Analyses testing environmental factors for the influence on the occurrence of particular vertiginid species on the sampled wetlands.

Species	Factors	LambdaA	F	P	Relation
<i>C. edentula</i>	<i>U. dioica</i>	0.01	5.95	0.040	+
<i>V. substriata</i>	shading	0.06	55.89	< 0.001	+
	water level	0.02	17.62	0.001	-
	<i>T. palustris</i>	0.03	25.29	0.002	+
	<i>C. acutiformis</i>	0.00	4.61	0.004	+
<i>V. pygmaea</i>	water level	0.04	21.22	< 0.001	-
	shading	0.03	21.94	< 0.001	-
	<i>U. dioica</i>	0.02	10.22	0.005	+
<i>V. moulinsiana</i>	litter moisture	0.10	51.23	< 0.001	+
	water level	0.03	15.03	< 0.001	-
	<i>C. acutiformis</i>	0.02	11.42	0.002	+
	<i>L. vulgaris</i>	0.01	6.59	0.025	+
	<i>T. palustris</i>	0.01	5.74	0.015	-
<i>V. angustior</i>	litter moisture	0.17	92.73	< 0.001	+
	water level	0.04	23.79	< 0.001	+
	<i>T. palustris</i>	0.01	10.40	0.007	+
	shading	0.01	4.69	0.048	-

TABLE 6. Results of variance partitioning (percentage of explained variation, F- and P-value) of canonical correspondence analysis (CCA) models relating the population density of five vertiginid species to local environmental variables and plant species abundance/share/plant composition. Local environmental variables [E] include moisture of litter, air humidity, ground water level and insolation. Percentage cover of ten most common plant species was used as variables describing vegetation ([V]). [E+V] = total variation explained by variables describing local environment and vegetation; [E] = variation explained by environmental variables; [V] = variation explained by vegetation variables; [E|V] = pure environmental variation; [V|E] = pure variation explained by vegetation; [E∩V] = variation shared by environmental and vegetation variables.

	Variance explained (%)	F	P
[E+V]	9.0	10.251	< 0.0002
[E]	7.6	14.279	< 0.0002
[V]	2.5	6.502	< 0.0002
[E V]	6.5	12.415	< 0.0002
[V E]	1.4	3.925	0.0010
[E∩V]	1.0		

Waldén, 1981), with moisture as the one of the most influential (Boycott, 1934; Cameron, 1970; Tattersfield, 1990; Wardhaugh, 1995; Pearce, 1997; Willing et al., 1998; Killeen & Moorkens, 2011). This is in accordance with the fact that particular vertiginid species can occupy a wide range of plant communities across their geographical ranges (papers cited in Speight et al., 2003). Thus, at the wide scale, hydrological conditions at their sites should be more important than the structure of vegetation.

On the other hand, studies conducted in western Carpathians by Horsák & Hájek (2003) had shown that there is a strong predictive value of vegetation on composition of molluscan communities in fens. They inferred that the vegetation reflects other abiotic and biotic factors affecting molluscan species, such as the effects of geographical position, geomorphology, climate and individual site histories. Additionally, surveys of Karlin (1961) have shown strong correlation between land snail species richness and vegetation cover. Nevertheless, the impact of individual plant species on the occurrence of snail species in the microhabitat has never been tested. The influence of other

biotic factors, for instance predation (Rosin et al., 2011), remains unknown in the case of wetland vertiginids.

The results of our study show that abiotic factors have a superior role in shaping the molluscan assemblages at a micro-scale. The results of the CCA general model show that habitat humidity is the major factor influencing the snail assemblages. However, the models performed for each locality separately indicate that differing factors play the primary role in shaping malacocenoses studied. On the Pliszka locality, the moisture fluctuations appear to be less important compared to the Ilanka locality. Under the relatively stable humidity conditions typical for this site, other factors – in this case shading – begins to play the primary role in shaping the snail assemblages. Thus, our results support the hypothesis that different factors may be important at different sites even when macroenvironmental factors are very similar (Juříčková et al., 2008), in accordance with the classical Shelford's (1931) law of tolerance.

Microhabitat Preferences of Vertiginid Species Studied

According to our results, *V. angustior* is a hygrophilous species occurring in plots with relatively high litter moisture and ground water level, often covered by *T. palustris*. This is in contradiction to most of the other authors describing the species as intolerant to habitats that are prone to flooding (Killeen, 2001; Cameron, 2003; Cameron et al., 2003; Książkiewicz, 2008; Killeen & Moorkens, 2011). However, dead leaves of *T. palustris* form specific, thick and freely drying litter which can provide a refuge when the water level is high. Negative affinity to shaded microhabitats shown by our analyses confirms that the known habitat preferences of the species (Cameron, 2003; Moorkens & Gaynor, 2003; Proschwitz, 2003), are important also at the micro-scale.

The presence of *Vertigo moulinsiana* is negatively affected by ground water level in the studied locations. These results are contrary to earlier studies from England (Killeen, 2003; Tattersfield & McInnes, 2003). On the other hand, abundance of *V. moulinsiana* was positively correlated with litter moisture on both studied localities. Notably, throughout the summer time the species was found mainly in the litter, while the previous studies had shown it climbs on plants during this period (Stebbing & Killeen,

1998; Killeen, 2003; Killeen & Moorkens, 2003). The preferences of *V. moulinsiana* to the relatively high water level reported by other authors could be therefore an effect of its affinity to the high ambient humidity. When the litter moisture is high (as in our study), presence of a high water table is no longer necessary to sustain the correct humidity in the microhabitat and the species is not forced to climb up the waterlogged plants. This hypothesis is supported by the observed high aggregation of *V. moulinsiana* in the less humid Ilanka locality, where the species occurred only in the plots with the highest litter moisture. Observed affinity of *V. moulinsiana* to *C. acutiformis* and *L. vulgaris* is most probably caused by the similar abiotic preferences of these plant and snail species.

Vertigo substriata was associated with shaded plots. Microhabitat preferences of this species are therefore consistent with its known habitat affinities, since the species is frequently found in forests (Pokryszko, 1990, 2003; Proschwitz, 2003). Additionally, the species occurs in microhabitats with high *T. palustris* and *C. acutiformis* abundance, which could be connected with its preferences to a specific type of litter (e.g., freely drying) or dense vegetation providing right shading in case of absence of trees.

Vertigo pygmaea prefers microhabitats with low water level and shading. As a facultative xerophile (Boycott, 1934) this species is less vulnerable to desiccation than the other vertiginid species. Therefore, *V. pygmaea* occupies plots more exposed to desiccation during the dry periods than other species studied.

Columella edentula occurred in microhabitats with high abundance of the nettle, *U. dioica*. Moreover, it frequently co-occurred with *V. pygmaea* in Ilanka locality and inhabits the less moisture plots on both locations. The species seems to be adapted to less humid microhabitat as eggs of *C. edentula* are probably calcified (Myzyk, 2005, 2011). As it is the most aggregated species in the Pliszka locality and the most uniformly distributed species in Ilanka, it seems to avoid plots with high water level and to be more robust to changes in litter moisture. Such fluctuations can be compensated for by the climbing behavior characteristic for this species (Piskorz & Urbańska, 2007; Książkiewicz, 2010) which was also observed in the locations studied. This behavior therefore could be an evidence of species-specific requirements to air humidity. The other explanation for the

climbing behavior suggested in the literature is thermoregulation (Cowie, 1985; Moreno-Rueda, 2007).

On the two localities studied we found differences in the composition of vertiginid malacofauna as well as in the distribution of particular species. Statistical analysis had shown this diversity is primarily caused by abiotic factors. The general model indicated that moisture is the principal factor influencing the species assemblages. Nevertheless, the hierarchy of the specific factors may vary depending on habitat and perhaps on season.

We have also shown the differences in preferences of the studied species to a range of abiotic and biotic factors. Because of the crucial role of abiotic factors, the influence of particular plant species is probably connected with providing specific refugia as well as influencing the abiotic traits of an environment.

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ISOLATION OF ORGANIC MATRIX NACREOUS PROTEINS FROM *HALIOTIS DIVERSICOLOR* AND THEIR EFFECT ON *IN VITRO* OSTEOINDUCTION

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ABSTRACT

The organic matrix proteins of molluscan nacre are known to engage biocompatible and osteoinductive properties. In this study, we aimed to extract *Haliotis diversicolor* nacreous proteins and assayed their effect on pre-osteoblastic cell differentiation. We found that acid extracted nacreous proteins (AEP) consisted primarily of a major protein band of 25 kDa and three other minor proteins. AEP enhanced alkaline phosphatase (ALP) activity of MC3T3-E1 cells both in time- and concentration-dependent manners. Transcriptional up-regulation of osteogenic markers, including collagen type I (COL-I), osteopontin (OPN) and osteocalcin (OCN), was also apparent in days 7 and 14 upon AEP treatments. At the translational level, higher protein expression of COL-I was evident in AEP treated cells, and the protein was presumably laid down as extracellular matrix. Further *de novo* sequencing of an AEP major protein revealed a match with the abalone mantle protein sometsuke. Conclusively, we demonstrated that *H. diversicolor* AEP contains a factor, potentially the mantle protein sometsuke, which may impart in the osteoblastic cell differentiation.

Key words: *Haliotis diversicolor*, nacre, bone, cell differentiation, osteogenesis.

INTRODUCTION

Bone is a mineralized connective tissue containing osteocytes, extracellular matrix and deposited minerals. In order to maintain bone integrity, a fine balance in bone formation and remodelling is required (Freemont, 1993; Hill, 1998; Katagiri & Takahashi, 2002; Proff & Romer, 2009; Raggatt & Patridge, 2010). Nevertheless, the activation of bone formation by osteoblasts has been viewed as the primary regimen to improve bone mass and bone architecture during bone repair (Horton, 2003; Caetano-Lopes et al., 2007; Lee et al., 2008; Suomi et al., 2008). Therefore, much research has been focused on controlling osteoblast differentiation using naturally extracted biomaterials to replace many known chemical stimulants, such as ascorbic acid and β -glycerophosphate (Rousseau et al., 2003, 2008). Osteoblast differentiation is under the control of central (hormonal and neuronal) and local (growth factors and cytokines) signalling (Komori et

al., 1997; Komori, 2000; Canalis et al., 2003; Zamurovic et al., 2004; Caetano-Lopes et al., 2007; Grabowski, 2009). In particular, the TGF- β superfamily plays many crucial roles in cell proliferation, differentiation, apoptosis, adhesion, migration and extracellular matrix protein production. The most potent local factors that belong to the TGF- β superfamily, bone morphogenetic proteins (BMPs), are also known to regulate osteoblast differentiation. BMPs bind to type I and II BMP receptors, which are transmembrane serine/threonine kinases and further phosphorylate Smad 1, 5 and 8. As a consequence, the binding complex translocates into the nucleus together with Smad 4 and regulates transcription of the targeted genes (Yamaguchi et al., 2000; Canalis et al., 2003; Chen et al., 2004; Kahai et al., 2004; Zamurovic et al., 2004; Suomi et al., 2008; Proff & Romer, 2009).

Recently, many experimental studies in osteoblast differentiation have relied on *in vitro* testing of the cultured cells, with one well-established

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in vitro model being a mouse calvarial cell line, MC3T3-E1. In response to the known inducers, these cells demonstrate an increase in alkaline phosphatase activity and up-regulation of collagenous and non-collagenous gene transcription and translation (Yamaguchi et al., 2000; Almeida et al., 2001; Kahai et al., 2004; Zamurovic et al., 2004; Carinci et al., 2005).

Nacre, or mother-of-pearl, is a natural biomaterial consisting of organic matrix proteins embedded in aragonite tablets. The organic matrix has been classified into soluble and insoluble matrices depending on their ability to solubilise in water (Marin & Luquet, 2004; Rahman et al., 2006). The soluble matrix controls aragonite crystal carbonate formation and deposition (Feng et al., 2000; Thompson et al., 2000; Zhang et al., 2006; Gries et al., 2011). In molluscs, bio-mineral complexes are essential for exoskeleton formation, the composition of which is comparable to that of vertebrates where the biominerals are crucial materials of endoskeleton (Wilt et al., 2003; Omelon et al., 2009; Lefebvre & Bhattaram, 2010). The significance of biomaterials is not only fundamental for skeletal biosynthesis, but their stimulatory and self-repairing capacity is also recognized as one of their bioactive properties. In the past decade, strong evidence from numerous shell studies has indicated that the soluble fraction of organic matrix extracts contain crucial factors to induce osteogenesis, both *in vitro* and *in vivo* (Lopez et al., 1992; Atlan et al., 1997, 1999; Lamghari et al., 1999; Almeida et al., 2001; Moutahir-Belqasmi et al., 2001; Rousseau et al., 2008). It is believed that diffusible substances from a piece of nacre inserted into a site of bone fracture can stimulate bone repair without showing any inflammatory side effect, providing excellent evidence for *in vivo* osteogenic activity of nacre (Atlan et al., 1999; Lamghari et al., 1999). Apart from the osteogenic property, some small extracted nacreous proteins inhibit cathepsin K, a protease that is secreted by osteoclasts during bone resorption (Duplat et al., 2007a, b).

In this study, we postulated that an osteoblast differentiation factor may be present in an acid-extracted nacreous protein (AEP) from abalone shell that plays a role in inducing a downstream cascade of gene regulation involved in osteoblast differentiation. Using the MC3T3-E1 cell line as an *in vitro* model, we found that AEP provoked an increased alkaline phosphatase activity and transcriptionally up-regulated many osteoblast markers, and enhanced secretion of

a ground-matrix protein for mineral deposition. The major protein isolated from AEP was a sometsuke-like protein, which may be responsible for induction of osteogenesis. This study fundamentally enhances our understanding of an invaluable biomaterial with high potential for future therapeutic applications.

MATERIALS AND METHODS

Extractions of Nacreous Proteins and Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Abalone (*Haliotis diversicolor* Reeve, 1846) shells were kindly provided by the Phuket Abalone Farm, Phuket, Thailand. The outer surface of the shells were cleaned by thorough brushing with metal brush or sandpaper followed by extensive washing with ultrapure water. Shells were either fragmented into small pieces (3–10 mm) or ground into fine powder. Two demineralization methods were used for extraction: (1) 10% acetic acid and (2) 100 mM EDTA. The fragments or powder were continuously stirred with demineralizing agents for 24 h at 4°C and were subjected to centrifugation (4,000 × g, 4°C, 20 min) to get rid of remaining debris. Thereafter, the supernatants were collected and dialysed with a 12-kDa cut-off membrane (Spectrum Laboratories, Breda, The Netherlands). The retentate was pooled and partially purified using two-step MicroCon centrifugal devices (Millipore, Billerica, Maryland, USA) with 50 and 100 kDa cut-off. This method allowed us to collect three sub-fractions: (I) < 50 kDa; (II) 50–100 kDa, and (III) > 100 kDa. Protein concentration was determined using a Bradford protein assay (Bradford reagent; Sigma-Aldrich, St. Louis, Missouri, USA) and measured at 595 nm.

For protein profiling, SDS-PAGE was performed according to methods described by Laemmli (1970). Approximately 10–15 µg of the fractionated proteins were loaded into the well and separated by 12.5% SDS-PAGE under reducing condition. Gels were either stained with Coomassie Brilliant Blue G-250 or subjected to silver staining (Mortz et al., 2001) using Proteo-Silver Silver staining kit (Sigma-Aldrich).

Cell Culture and Treatment

An MC3T3-E1 cell line (passage 16; ATCC, CRL-2593, USA) was initially propagated in an alpha minimum essential medium (α-MEM;

Gibco, Grand Island, New York, USA) supplemented with 2 mM L-glutamine, 10% fetal calf serum and 100 µg/ml penicillin/streptomycin. Treatment conditions on cultured cells followed the methods described by Lao et al. (2007). Culture cells were propagated until approaching confluence. They were subsequently subcultured and plated into 4-well plates at a density of 2,500 cells/well in the humidified atmosphere at 5% CO₂ with the culture medium changed every three days. Testing substances were added every two days within a 14-day period according to these following conditions: (1) culture cells maintained in a Dulbecco's modified Eagle's medium (DMEM; Invitrogen, Carlsbad, CA, USA) as "sham control", (2) cells treated with osteoinductive medium containing 50 µg/ml ascorbic acid in 10 mM β-glycerophosphate (Sigma; St.Louis, Missouri, USA) as "positive control" (Rousseau et al., 2003) and (3) cells treated with 50 and 100 µg/ml acid extracted proteins (AEP) which were resuspended in DMEM medium. After 14 days, the cells in each individual culture well were harvested to monitor their osteogenic activities at both the transcriptional and translational levels as described below.

Measurement of Alkaline Phosphatase (ALP) Activity

ALP activity was determined on the cultured cells at days 1, 7 and 14 post-AEP treatments. Cultured cells were mechanically detached from the cultured wells and washed with ice cold PBS. The ALP activity was determined with the cell lysates based on formation of *p*-nitrophenol (PNP) using the *p*-nitrophenylphosphate kit (Spinreact, St. Esteved'enBas, Girona, Spain) following manufacturer's instructions.

The optical density of PNP was measured at 405 nm using a microplate reader. The specific ALP activity was expressed as µmole PNP/min/mg protein calculated from independently triplicate experiments. Statistical analysis between each group was computed by a paired Student's *t*-test and a *P* value < 0.05 was considered statistically significant.

Analysis of Osteogenic Transcripts using Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

MC3T3-E1 cells cultures were trypsinized on days 1, 7 and 14 for total RNA isolation using Trizol reagent (Invitrogen, Carlsbad, California, USA). First-strand cDNA was synthesized with 1 µg of total RNA using a SuperScript™ III Reverse Transcriptase (Invitrogen) and oligo-dT according to the manufacturer's protocol. Forward and reverse primers of mouse osteogenic markers including collagen type I (COL-I), osteopontin (OPN), osteocalcin (OCN) and their expected product size were listed in Table 1. GAPDH mRNA was used as an internal control. For PCR amplification, the PCR conditions included a cycle at 94°C (5 min), 30 cycles at 94°C (30 sec each), 2 cycles at T_m (see Table 1) for 30 sec and at 72°C (1 min), and a final step at 72°C (10 min). Densitometric analysis of RT-PCR products in the same, pixel area was performed using ImageJ software (NIH, Bethesda, Maryland, USA) and the data were expressed as mean ± S.D. calculated from triplicate experiments. Statistical analysis of the expression levels within different days of treatments and among the treatment groups in each treatment period was performed using ANOVA. Statistical difference was considered at the *P* value ≤ 0.5.

TABLE 1. Primers for osteogenic markers used in PCR and their expected product size.

Primer	Sequence (5'-3')	T _m (°C)	Product size (bp)
Collagen type IF	ACTGGTACATCAGCCCGAAC	62.4	476
Collagen type IR	GGTGGAGGGAGTTTACACGA	62.4	
Osteopontin F	CGATGATGATGACGATGGAG	60.4	434
Osteopontin R	TCTCCTGGCTCTCTTTGGAA	56.3	
Osteocalcin F	TATGTGTCCTCCGGGTTCAT	60.4	455
Osteocalcin R	GCCCTCTGCAGGTCATAGAG	64.5	
GAPDH F	AACTTTGGCATTGTGGAAGG	58.4	472
GAPDH R	CCCTGTTGCTGTAGCCGTAT	62.4	

Immunofluorescence and Confocal Laser Scanning Microscopy

MC3T3-E1 cells were cultured as described above with round coverslips placed on the bottom of the wells. During cell treatments with different testing agents, cells in each well were washed with PBS at days 7 and 14. They were then fixed with 4% paraformaldehyde in phosphate buffer overnight and rinsed with PBS three times. Fixed cells were further permeabilized with cold methanol for 30 sec and transferred to PBS. The following steps were carried out in a moist chamber at room temperature. Cells were blocked with 5% normal goat serum (NGS) and incubated (90 min) with rabbit anti-collagen type I (Merck KGaA, Darmstadt, Germany) at 1:1000 dilution in PBS containing 2% NGS. After rinsing in PBS several times, cells were further incubated (60 min) with Alexa 488 conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, Oregon, USA) at a dilution of 1:500. Cell nuclei were counter-stained with TO-PRO-3 (Molecular Probes) at 1:1000 dilution and mounted with glycerol/PBS (1:1, v/v). The fluorescent images were acquired by a FV1000 confocal laser scanning microscope (Olympus, Tokyo, Japan) using argon (excitation wavelength = 488 nm) and krypton (excitation wavelength = 633 nm) lasers and a Kalman's line-by-line scanning mode.

Reverse Phase High Performance Liquid Chromatography (RP-HPLC) and Mass Spectrometry (MS/MS)

The partially purified AEP was resuspended with 0.1% TFA and subjected to RP-HPLC (Discovery BIO Wide Pore C5 column; 15 cm x 2.1 mm) using a two-step linear gradient (0–5% CH₃CN containing 0.1% TFA in 5 min, then 5–60% CH₃CN containing 0.1% TFA in 90 min at a flow rate of 0.2 ml/min). Selected fractions were pooled, lyophilized, and repurified using different gradient conditions (0–39% CH₃CN containing 0.1% TFA in 10 min, then 39–50% CH₃CN containing 0.1% TFA in 60 min at the flow rate of 0.2 ml/min). Proteins were detected at 210 nm. Selected fractions were collected, lyophilized and subjected to in-solution tryptic peptide digestion for overnight. The peptides were desalted, concentrated in 0.1% formic acid and 2% acetonitrile and further analysed by one-dimensional (1D) LC MS/MS and N-terminal sequencing at the Australian Proteome Analysis Facility (NSW, Australia).

RESULTS

Profiles of Partially Purified Nacreous Proteins

We compared protein yields among four nacreous protein extraction methods from *Haliotis diversicolor* shells which were based on physical (powder and small chips) and chemical demineralization (acetic and EDTA) methods. As shown in Table 2 shells ground into powder and extracted with acetic acid (AEP) appeared to give the highest yield of protein of ~37 mg/kg dry weight powder. Therefore, this extraction method was selected for further purification and used for experiments in this study. Our preliminary data of the partial purification using two-step cut-off (50 and 100 kDa) centrifugation

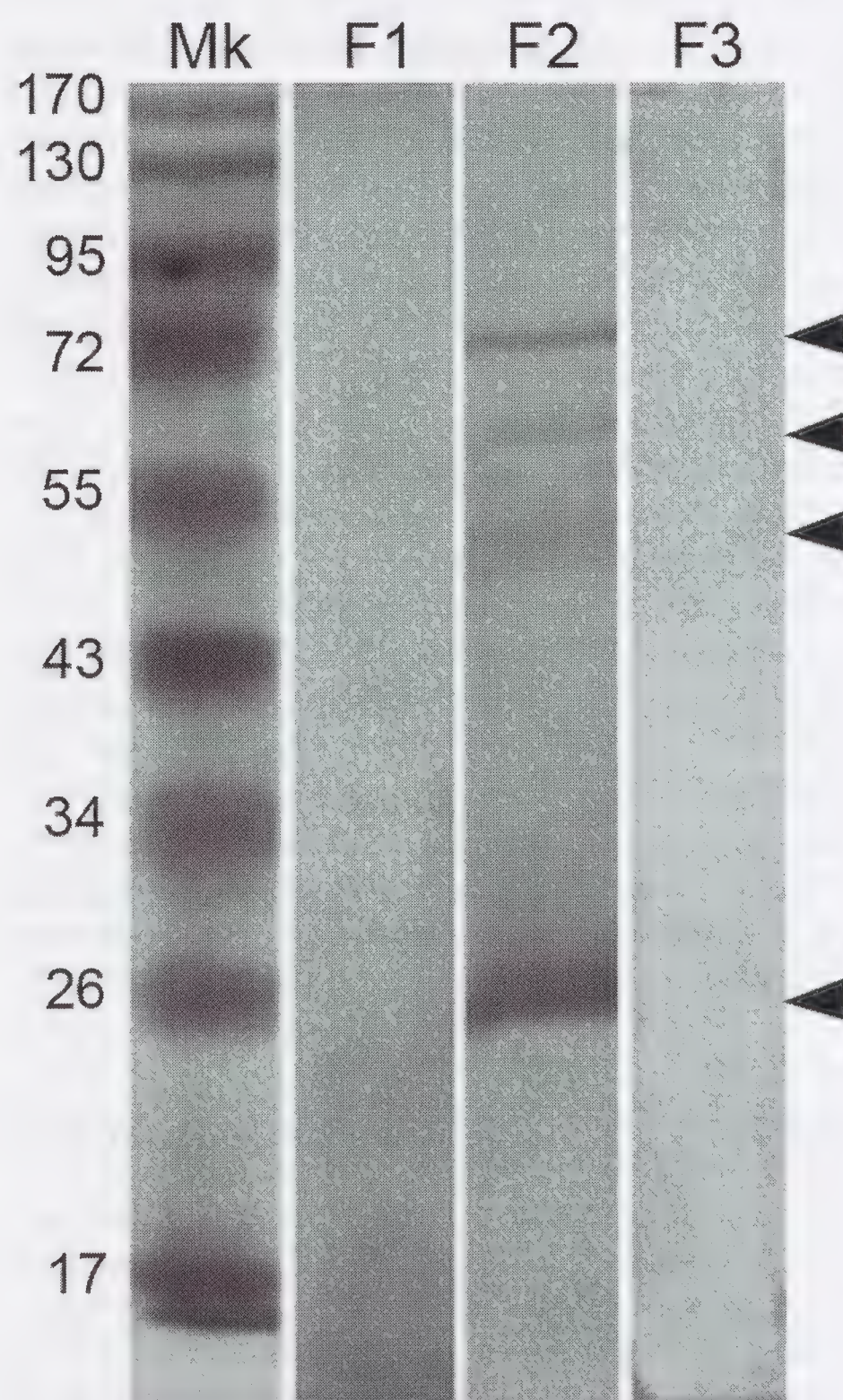


FIG. 1. SDS-PAGE and silver stain profiles of the partially purified *H. diversicolor* acid nacre protein extracts (AEP) by two-step filtration centrifugation. The three fractions shown are F1: < 50 kDa cut-off, F2: 50–100 kDa and F3: > 100 kDa. Arrowheads indicate major proteins and their molecular masses. Mk: standard marker.

TABLE 2. Relative nacreous protein concentration and protein ratio in dry weight by different physical and demineralization methods.

Extraction method	Relative protein concentration (mg/ml)	Protein ratio in dry weight (mg/kg)
Powder + Acetic acid	0.56	37
Fragments + Acetic acid	0.51	34
Powder + EDTA	0.46	31
Fragments + EDTA	0.43	29

indicated that the majority of nacreous proteins were found in fraction 2 (Fig. 1, lane F2). Therefore, we chose to conduct experiments exclusively with F2 in this study. The profile of AEP in F2 as revealed by silver staining showed a prominent protein band at 25 kDa and additional faint bands at 52, 60 and 72 kDa of much lower intensity (Fig. 1). Coomassie Blue staining of these partially purified AEP revealed only one major band at a 25 kDa (data not shown), indicating that the 25 kDa band is of the major protein in this extraction methodology.

AEP Enhanced Alkaline Phosphatase (ALP) Activity

To evaluate the effect of AEP on the pre-osteoblast MC3T3-E1 cells, we compared enhancement of ALP activity, one of the well-known markers for osteoblast differentiation. Generally, ALP activity gradually increased in a time- and concentration-dependent manner when treated with osteoinductive medium (positive control) and AEP, compared with the control groups (Fig. 2). At day 1, ALP activity

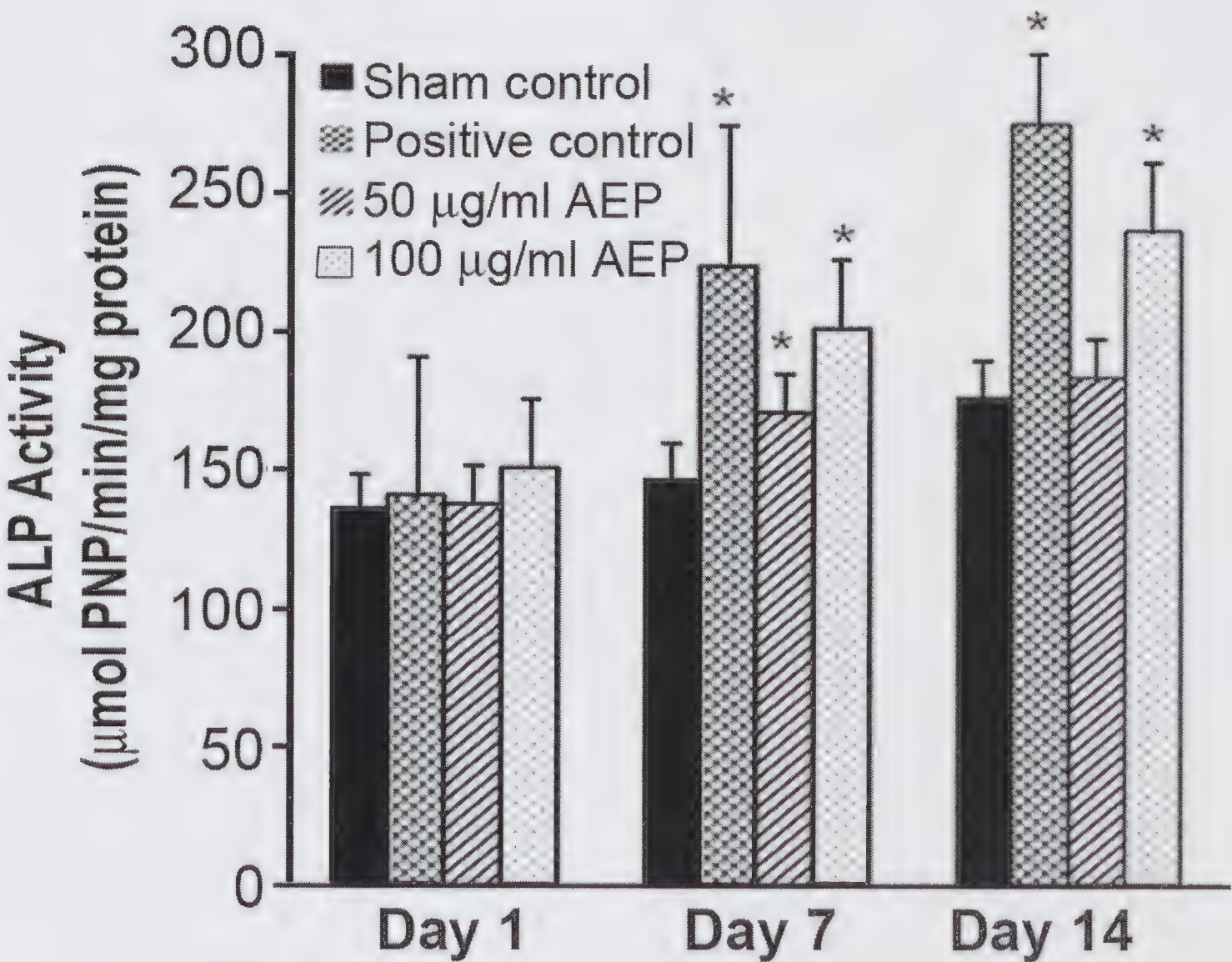
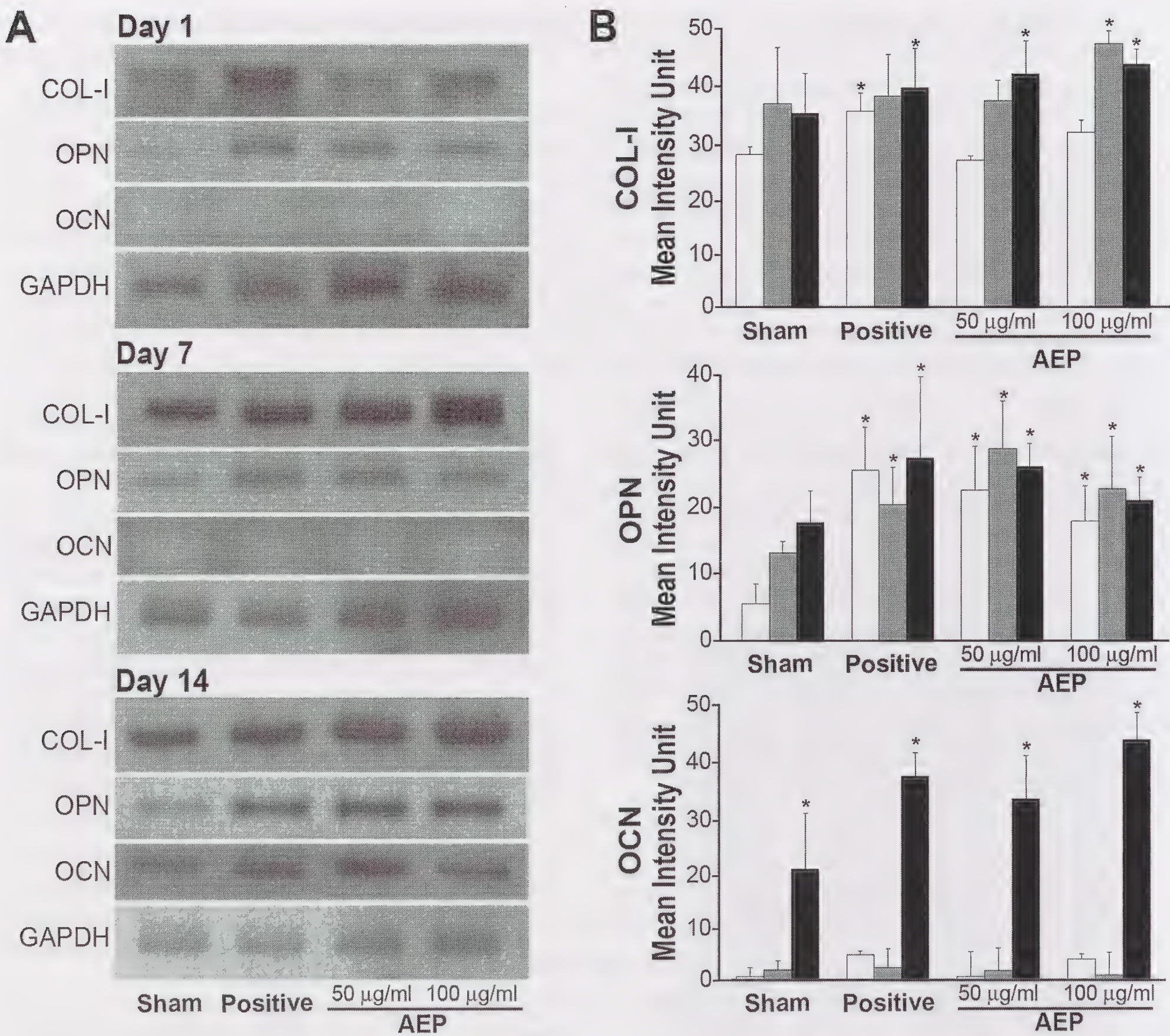


FIG. 2. Time-course and concentration-dependent effect of AEP on alkaline phosphatase activity in MC3T3-E1 cells. Cells were treated with DMEM (sham control), 10mM β-mercaptoethanol and 50 μg/ml ascorbic acid (positive control), 50 and 100 μg/ml AEP. Results are expressed as mean ± S.D calculated from triplicate experiments. *indicates significant difference ($P < 0.05$) to control treatment within analysis day.



was relatively comparable in all treatment and control groups. However, the activity was significantly ($P < 0.01$) enhanced in day 7 and was even more pronounced in day 14, particularly in 100 µg/ml AEP and positive control groups. The highest ALP activity in 100 µg/ml AEP-treated cells at day 14 was 236.02 ± 0.33 U/L/min/mg protein, which was slightly less than positive control (275.07 ± 0.24 U/L/min/mg protein) but significantly greater ($P < 0.05$) than 50 µg/ml AEP (183.46 ± 0.33 U/L/min/mg protein) or the sham control (176.85 ± 0.13 U/L/min/mg protein).

Up-regulation of Osteogenic Markers' Transcripts following AEP Treatment

In order to investigate the effect of AEP on osteoblast differentiation, RT-PCR and densitometric quantitation of the amplified products including COL-I, OPN, and OCN was performed (Fig. 3). Following seven days of MC3T3-E1 cell cultivation, COL-I gene expression was up-regulated in all groups studied. In particular, the COL-I band density in 100 µg/ml AEP-treated cells (50.14 arbitrary density units) was apparently higher than the other groups and

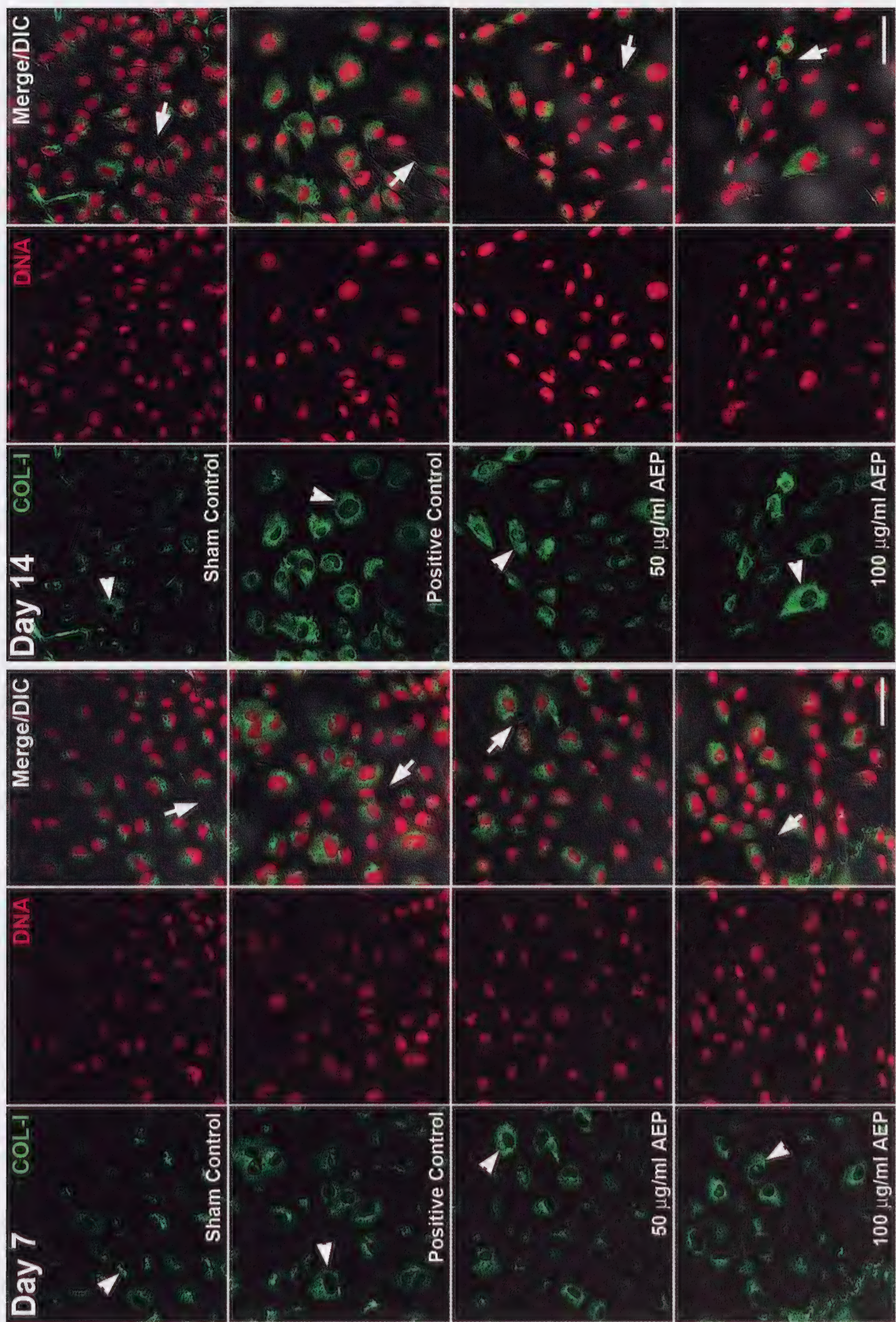


FIG. 4. Immunofluorescence and confocal microscopy of collagen type I (COL-I) expression in MC3T3-E1 cells treated with AEP at days 7 and 14. In each panel, green fluorescence represents COL-I staining (left column), red fluorescence represents DNA staining by TO-PRO 3 (middle column), and the right column is merged fluorescent micrographs with DIC. Note the crescent-like staining pattern in sham control but spreading throughout cytoplasm in AEP-treated cells (arrowheads). Arrows indicate the fibrillar structure of the extracellular matrix.

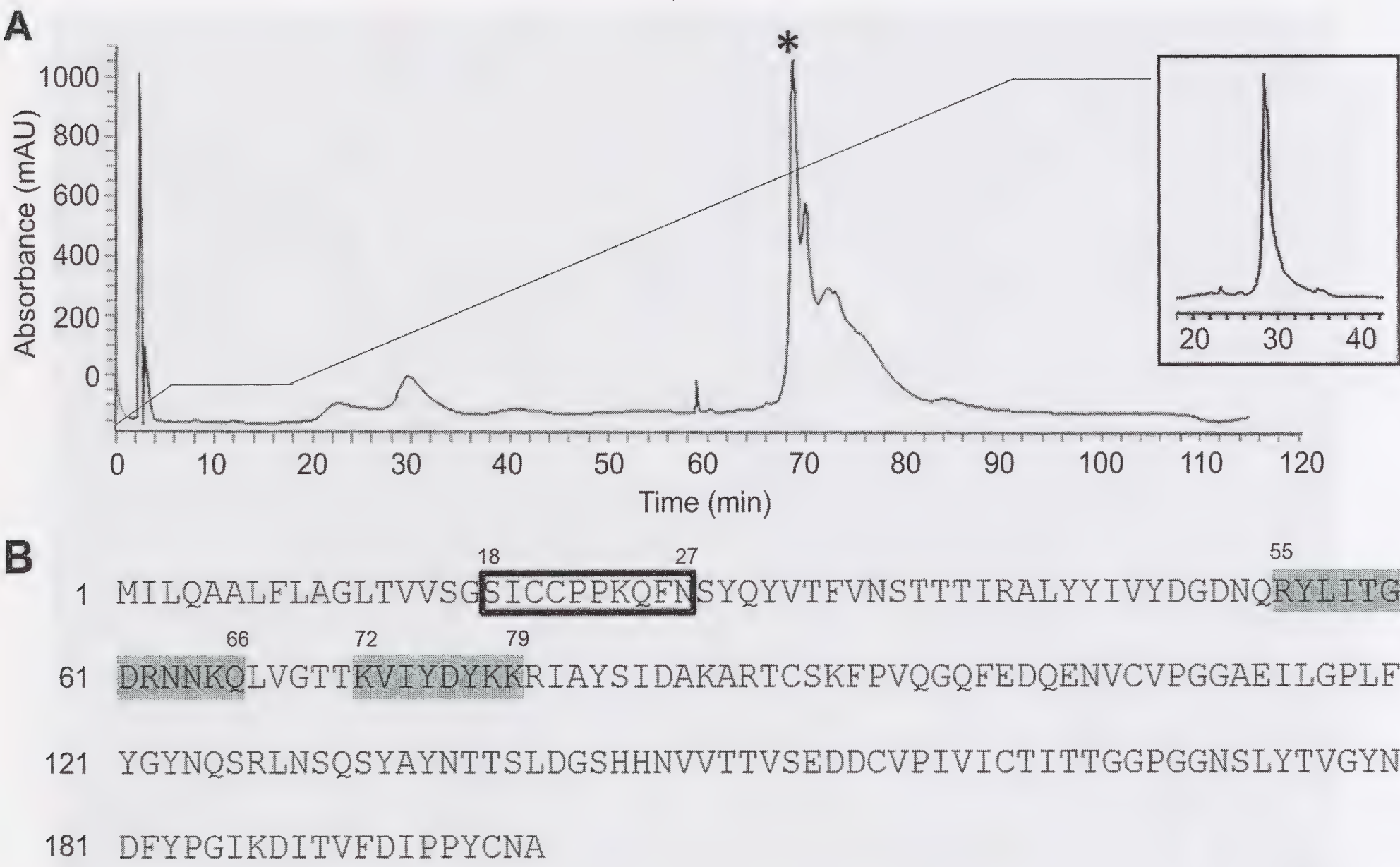


FIG. 5. Purification and sequencing of AEP 25 kDa protein from *H. diversicolor*. A: Representative chromatogram of primary RP-HPLC purification with the major 25 kDa peak indicated by an asterisk which was re-purified (inset) for sequencing; B: Sometsuke protein sequence of *H. asinina* showing the sites matching the *H. diversicolor* AEP 25 kDa protein as determined by 1D MS/MS spectra (shaded in grey) and N-terminal sequencing (boxed).

kept relatively constant towards day 14 (52.63 units). This similar trend of increased COL-I gene expression was also observed in the 50 µg/ml AEP-treated and positive control groups. The expression of OPN, a non-collagenous marker for osteoblast differentiation, was only at the detectable level on day 1 of the sham control (8.47 units). However, upon AEP treatment even at day 1, the OPN transcriptional level was greatly enhanced by ~3 folds in both 50 and 100 µg/ml AEP-treated cells. At day 7, the increase in band intensity was relatively low compared with day 14, where the band intensity peaked up to 77.9 units in both 50 and 100 µg/ml AEP treatments. Positive control cells constantly displayed high OPN transcription in MC3T3-E1 cells over the three period of culture. OCN transcription, another specific and late marker for osteoblast differentiation, was undetectable at day 1 or day 7. However, its transcriptional level was greatly enhanced at day 14 in all groups with band intensity of ~21–26 units that were ~1.4–1.8 fold higher than that detected in the sham control.

Enhancement of COL-I Protein in Pre-osteoblasts Upon AEP Treatments

To test whether AEP treatments also stimulated COL-I expression at the protein level, MC3T3-E1 cells were subjected to anti-COL I immunohistochemistry and their staining distribution compared at days 7 and 14. As shown in Figure 4, cultured cells showed an intense crescent-like staining pattern (arrowheads) over one side of the nuclear region which was similar in both days 7 and 14 (left column in each panel). Upon treatment with AEP (both 50 and 100 µg/ml; third and forth rows), anti-COL I staining spread throughout the cytoplasm of most cells in addition to the crescent-like pattern which was comparable to the pattern observed in the positive control (second row). The lay-down of ground matrix between the cellular spaces as seen by differential interference contrast (DIC) microscopy (second and forth rows) was quite apparent, particularly positive control and 100 µg/ml AEP treatment (arrows).

Identification of a Major Protein Sequence in AEP Extract

The AEP extract used in this study was further purified by reversed-phase HPLC and characterized by 1D LC-MS/MS and *N*-terminal sequencing. Initially, three protein peaks were obtained: a major peak at 65–80 min, and two minor peaks at 20–34 and 58–60 min (Fig. 5A). The major peak was re-purified to provide a single peak (Fig. 5A, inset) and further subjected to sequence analysis. Following 1D LC-MS/MS, the MASCOT search engine was used for protein identification by comparing the mass spectra of the fragmented peptides (2+ up to 4+ charged) with the proteins present in the NCBI database. Two peptides matched with *Haliotis asinina* Linnaeus, 1758, sometsuke (GenBank accession number DW986219) (Fig. 5B). The peptide sequences matched at positions 55–66 (12 amino acids) and 72–79 (eight amino acids). In addition, *N*-terminal sequencing confirmed that the 10 amino acids at position 18–27 were identical to the sometsuke protein sequence. This AEP sequence analysis thus suggests that sometsuke is a major part of the organic matrix in *H. diversicolor* nacreous layer that may induce osteoblast differentiation.

DISCUSSION

In our study, we used mechanical- and chemical-based methods to isolate proteins from the *H. diversicolor* nacreous layer and showed that AEP extraction was the most effective method in nacreous protein isolation while it still maintained the biological function of the extracted proteins in stimulating osteoblast differentiation. This is consistent with studies on *Pinctada maxima* (Jameson, 1901), where osteogenic activity exists within the soluble organic matrix of nacre (Lamghari et al., 1999; Almeida et al., 2001; Moutahir-Belqasmi et al., 2001; Mouriès et al., 2002; Rousseau et al., 2003, 2008). At a first step towards isolating the osteogenic differentiation factors, we showed that 10% acetic acid gave the highest extracted protein concentration (0.56 mg/ml) equivalent to 37 mg dry nacreous protein/kg nacre weight. This AEP extract showed a prominent protein band at 25 kDa, which was at a lower molecular mass than the cut-off molecular mass of the centrifugal device used for purification. One

explanation for this phenomenon is that this nacreous protein may be in a dimeric or multimeric form bound together by disulfide bridges or by electrostatic interaction as also reported for other nacreous proteins (Marin et al., 2005; Kong et al., 2009), which are subsequently disrupted by reducing or high salt conditions. This finding is consistent with the results reported by Bédouet et al. (2007), indicating the presence of low molecular weight molecules in the shell matrix. Interestingly, further purification of this AEP protein followed by partial peptide sequencing indicated that our AEP corresponded to the abalone mantle protein, sometsuke, a ~20 kDa protein expressed in the anterior zone of the outer fold of abalone mantle (Jackson et al., 2006). Its expression correlates with nacre and prism soluble matrix in *H. asinina*. Physiologically, it has been suggested that sometsuke possesses the ability to organize the shell matrix framework and has calcium binding properties (Marie et al., 2010). Besides, sometsuke has also been known to engage its function in shell pigmentation patterning in *H. asinina*, as shown by Jackson et al. (2006).

In fact, the proposed function of the sometsuke protein in calcium binding has drawn attention to the biomineralization function of shell organic matrix, the knowledge of which has gradually accumulated in other *Haliotis* species. Perlustrin, a 9 kDa nacre soluble organic matrix protein extracted from *Haliotis laevigata* Donovan, 1808, contains an insulin-like growth factor binding protein (IGF-BP) domain that enables IGF-binding, known to be crucial for cell interactions (Weiss et al., 2000). Other smaller nacreous proteins of *H. laevigata* include perlucin, an 18 kDa nacre soluble organic matrix protein that has a C-type lectin domain which facilitates calcium carbonate precipitation (Weiss et al., 2000). In *Haliotis rufescens* Swainson, 1822, lustrin A, a nacre insoluble protein of 142 kDa has been reported to adhere nacre tablets, assuming a function similar to that of muscle protein titin, an excitation-contraction coupling protein (Shen et al., 1997). The last two identified abalone aragonite proteins, AP7 and AP24, are moderately acidic and considerably novel, since they show no homology with any known proteins. While AP7 contains four cysteine residues involved in its conformation through disulfide bridging, AP24 contains an *N*-glycosylated domain which is believed to participate in calcium

binding (Michenfelder et al., 2003). Despite the fact that these abalone nacreous proteins play significant roles in biomineralization, most of them are related to organization of the nacreous layer within the shell. Unlike the studies reported in some oyster and abalone species, additional biomedical roles in osteoinduction both *in vitro* and *in vivo* have also been experimentally validated, making them potentially valuable pharmaceutical agents.

During *in vitro* osteogenesis, the three check points are generally considered including: (1) incremental ALP activity as an initial indicator of osteoblast differentiation (Hessle et al., 2002), (2) changes in osteoblast-specific gene markers throughout the cell differentiation process (Spreafico et al., 2006; Sila-asna et al., 2007) and (3) collagenous protein synthesis and its lay-down to become extracellular matrix (Li et al., 2011; Reffitt et al., 2003). In our study, we found that AEP at 100 µg/ml strongly increases ALP activity comparable to that of a positive control known to stimulate ALP activity (Son et al., 2007). This finding agrees well with the study in *Pinctada fucata* Röding, 1798, in which the mantle extract PFMG3 at 10 µg/ml considerably stimulates ALP activity following a 48 h incubation with MC3T3-E1 cells (Wang et al., 2011). In addition, water-soluble organic material (WSM, 1300 µg/ml) increases ALP activity in MRC5 cells when incubated for three days, and rat bone marrow stromal cells (135 µg/ml) when treated for seven days (Mouries et al., 2002). Notably, the concentration of AEP used in our study (at the microgram level) was in the same range as that used by other studies, suggesting a comparable physiological potency of our extracting compounds with other existing compounds. Inversely, Rousseau et al. (2003) found that WSM inhibited ALP activity, which may have occurred because of differences in the concentrations of WSM used to treat cells. In fact, we found that AEP at 200 µg/ml was rather toxic to MC3T3-E1 cells (data not shown).

We also found that AEP exerted up-regulation of gene markers (COL-I, OPN and OCN) related to osteoblast differentiation. Most notably, both 50 and 100 µg/ml AEP stimulated a relatively higher level of expression of COL-I and OPN after one day of culture. This appears to be a quicker response of MC3T3-E1 cells to AEP when compared with those treated with 200 µg/ml of ethanol soluble matrix (ESM)

extracted from oyster nacre (Rousseau et al., 2008). Concurrently, ESM enhances OPN transcription more than RP-HPLC fractions and the cells reach their maximum transcription level on day 21 (Rousseau et al., 2008). In the late stage of osteoblast differentiation concomitant with mineralization, osteocalcin is expressed (Caetano-Lopes et al., 2007). Our results revealed that OCN mRNA was present at day 14 but not at day 1 or 7. In contrast, the expression of OCN mRNA was reduced when cultivated with ESM and its HPLC fractions (Rousseau et al., 2008). Finally, we found that pre-osteoblasts were induced to translate higher levels of collagen type I by day 7 up to day 14 of culture, consistent with the positive control. This collagen is presumably laid down extracellularly by mature osteoblasts to become the most predominant component in the ground substances of the bone matrix (Caetano-Lopes et al., 2007).

In conclusion, our study has shown that AEP from *Halotis diversicolor* increased ALP activity, up-regulated all osteoblastic differentiation markers studied, and stimulated collagen ground substance lay-down *in vitro*. We believe that the organic extracts from nacreous layer of *H. diversicolor* constitute the signalling molecules, one of which could be a 25-kDa sometsuke-like protein, which play a role in promoting osteoblast differentiation and osteogenesis presumably through its calcium binding property. While the other function of sumetsuke protein in shell coloration patterning is also of particular interest in this species, however, it still requires further extensive investigations to address this question.

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DEVELOPMENT AND VALIDATION OF A SOLID-PHASE RADIOIMMUNOASSAY FOR MEASURING PROGESTERONE AND TESTOSTERONE IN OCTOPUS GONAD EXTRACTS

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ABSTRACT

Commercially available radioimmunoassays (RIA) with ¹²⁵I-labeled hormones were adapted, developed and validated to quantify progesterone and testosterone in gonad extracts of *Octopus maya*, considered an easily domesticated species with potential in aquaculture. Development of the RIAs was divided into four phases: (1) extraction of progesterone and testosterone from gonads, (2) preparation of gonad extracts for RIA, (3) standards preparation, and (4) RIA procedure. Hormones were extracted twice with 15 mL of diethyl-ether each time. The recovered hormone and the solvent effect on extraction were evaluated. RIAs were validated based on specificity, sensitivity, accuracy, precision and reproducibility. Serially diluted gonad extracts were parallel to the standard curves of each hormone, indicating specificity. Sensitivities for both hormones were less than 0.1 ng•g⁻¹, and did not change by more than 20% during the ¹²⁵I half-life. Accuracy was 102.25 ± 11.22% for progesterone and 81.62 ± 6.24% for testosterone. For both hormones, precision was close to 13% and reproducibility 17%. The precision/reproducibility ratio indicated a system with good stability. The amounts of the hormones recovered increased when the number of extractions was increased. Hormone concentrations also increased when the volume of diethyl-ether used per extraction was increased. The range of progesterone and testosterone concentrations quantified in this study is in agreement with the range found in other octopuses. Finally, the developed and validated RIAs meet the requirements for quantification of progesterone and testosterone in *Octopus* gonad extracts and show that it is possible to use ¹²⁵I-labeled hormone as well as the plastic coated-tubes from commercial RIA kits to analyse variations in hormone levels associated with gonad developmental stages and the reproductive cycle of octopuses, allowing exploration of their possible functions.

Key words: hormone, commercial RIA kits, specificity, sensitivity, accuracy, precision, reproducibility, *Octopus maya*.

INTRODUCTION

Steroids are widely distributed throughout the animal kingdom, where they act as regulators of numerous biochemical and physiological processes (Finch & Rose, 1995; Lafont, 2000; Hanoune, 2007; Köhler et al., 2007). Aquatic invertebrates are able to produce “vertebrate-type” sex steroid hormones with a distribution consistent with their known reproductive roles in

vertebrate reproduction, which supports the idea of the universality of steroidogenesis (Carreau & Drosowsky, 1977; Sandor, 1980; Lafont & Mathieu, 2007; Di Cristo et al., 2010; Treen et al., 2012). It is generally agreed that the hormonal control of reproduction in invertebrates is not as well documented as in vertebrates, and even less so for cephalopod molluscs (Lehoux & Sandor, 1970; Joosse, 1972; Lafont, 2000; Ketata et al., 2008; Kikuyama & Tsutsui, 2011).

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The presence of vertebrate-type steroid hormones has been documented in some invertebrate groups (Lehoux & Sandor, 1970; Lafont, 1991), but mainly in molluscs and echinoderms (Janer & Porte, 2007; Köhler et al., 2007; Lafont & Mathieu, 2007; Gust et al., 2010). In cephalopods, the reports are virtually restricted to a single species: *Octopus vulgaris* Cuvier, 1797. In many instances, the identification of these sex steroids relies only on radioimmunoassay (RIA) techniques. In fact, the RIA technique was the pioneer in the field with octopus and provided the preliminary evidence for the presence of “vertebrate-type” sex steroid hormones (i.e., progesterone, testosterone and/or 17 β -estradiol) in the reproductive system and particularly in the gonads of species of octopuses such as *Eledone cirrhosa* (Lamarck, 1798) (Nikitina et al., 1977; Young, 1992), *Octopus mimus* Gould, 1852 (Olivares-Paz et al., 2003), and *Octopus vulgaris* (D’Aniello et al., 1996; Di Cosmo et al., 2001). Coupled with RIA, other highly specific techniques as fluorometry (Nikitina et al., 1977), spectrophotometry (Nikitina et al., 1977) and HPLC (D’Aniello et al., 1996) have been used on octopuses as confirmative proofs. However, such proofs and others (e.g., gas chromatography or liquid chromatography coupled with mass spectrometric detection) require sophisticated equipment (Janer-Gual, 2005; Janer & Porte, 2007; Gust et al., 2010).

During the pioneering RIA studies using tritium labelled tracers to estimate progesterone and testosterone concentrations in octopus species (e.g., D’Aniello et al., 1996; Di Cosmo et al., 2001), the authors did not provide direct evidence of the reliability and validity of their method; nor did they investigate/optimize the extraction procedure. It is therefore difficult to use their protocols as a standardized method in order to assess progesterone and testosterone levels in cephalopod gonads. Of course, the validity and use of RIA is not considered novel; but RIA are still normally used as monitoring techniques in programs of octopus reproduction (Larson & Anderson, 2010), and there has been a growing interest in standardizing steroid hormone measurements by this immunological method due to its low cost and minimal hazards. Nevertheless, evaluation of analytical methods to measure sex steroid hormones in cephalopods requires reliability and validation; all the more so since the commercial kits are designed to be used in humans and not for the species in question

(Abraham, 1975; Cekan, 1975, 1979; Moss et al., 1976; Hafs et al., 1977; IAEA, 1984; Ezan et al., 1991; Chard, 1995).

Our aim was thus to adapt, develop and validate a commercially available solid-phase radioimmunoassay with ¹²⁵I-labelled hormones, as well as to describe a protocol of hormone extraction in order to measure progesterone and testosterone concentrations in octopus gonad extracts.

MATERIALS AND METHODS

Chemical and Reagents

All reagents were of HPLC grade. Diethyl-ether (CHROMASOLV® $\geq 99.9\%$ inhibitor-free), sodium phosphate dibasic, anhydrous ($\geq 98\%$, Fluka®), sodium phosphate monobasic, anhydrous ($\geq 99.0\%$, Fluka®), testosterone (purity $\geq 99.0\%$, Fluka®), and progesterone (minimum 99.0%, Sigma®) were from Sigma-Aldrich, Inc., Mexico. Methyl alcohol (J. T. Baker®) was from Mallinckrodt Baker, Inc., Phillipsburg, New Jersey, USA. Progesterone (DSL-3900) and testosterone (DSL-4000) coated-tube radioimmunoassay kits were purchased from Diagnostic Systems Laboratories®, Inc. Webster, Texas, USA.

Experimental Octopuses

Mature male ($n = 15$) and female ($n = 22$) *Octopus maya* Voss & Solís-Ramírez, 1966 (300–700 g body weight) used for validation of the RIA analysis were caught from January to May 2008 on the continental shelf of the Yucatán Peninsula in front of Sisal Harbor (21°9’55”N, 90°1’50”W). The octopuses were transported 300 m inland to the UMDI-UNAM laboratory; then kept in individual flow-through 80-L tanks with aerated seawater for two days without food to avoid feeding interfering with the physiological condition of the animals (Rosas et al., 2011). One PVC tube (10 cm in diameter) per octopus was placed in each tank as a refuge. Before dissecting the gonad, octopuses were immersed in seawater at 5°C for 3 min (derived from Roper & Sweeney, 1983) to induce a reduction in their metabolism (narcotisation), in consideration of ethics (Mather & Anderson, 2007) and their welfare during manipulations (Moltschaniwskyj et al., 2007), and it was performed in this way to avoid possible interference by chemical anaesthetics with the subsequent

TABLE 1. Steroid levels as determined by radioimmunoassay in *Octopus sp.** Authors within each source are ordered according to year of publication. The extraction procedure of steroid hormones from each tissue was performed two times, except where indicated by the symbol †. Refer to table 5 for the amount of solvent and the number of extractions.

Species	Tissue	Gonad development stages	Progesterone	Testosterone	Sources
<i>Eledone cirrhosa</i>	Ovary	third stage “mature”**	8.8	-	(Nikitina et al., 1977)
<i>Eledone cirrhosa</i>	Hemolymph	vitellogenesis	< 0.0013	< 0.000069	(Young, 1992)§
<i>Octopus vulgaris</i>	Testis	***	~3.3	~5.3	(D’Aniello et al., 1996)
	Hemolymph	***	~0.3	~0.4	
<i>Octopus vulgaris</i>	Ovary	Previtellogenesis	~0.030 to 0.040	-	(Di Cosmo et al., 2001)†
		early vitellogenesis	~0.040 to 0.045	-	
		full vitellogenesis	~0.200	-	
		late vitellogenesis	~0.020 to 0.025	-	
	Hemolymph	-	undetected	-	
<i>Octopus mimus</i>	Testis	Immature-maturing	~6.5 to 7.0	~7.2 to 8.5	(Olivares-Paz et al., 2003)
		mature	~7.0 to 8.2	~8.5 to 9.5	
		spawning	~3.5	~5.0 to 5.5	
<i>Octopus maya</i>	Ovary	mature	0.90 to 2.22	0.08 to 0.14	This study ^a
	Testis	mature	0.80 to 1.60	0.05 to 0.22	
<i>Octopus maya</i>	Ovary	mature	4.42 to 10.11	0.28 to 0.56	This study ^b
	Testis	mature	3.96 to 6.57	0.30 to 0.67	

*Concentration standardized to ng•g⁻¹ or ng•mL⁻¹.
**Considered as mature according to Arkhipkin (1992) and Boyle & Chevis (1992).
***Author indicated only adult males.
~Approximate values obtained from figures of each source.
-No data.
§Author indicated that hormonal level has little variation during the development of oocytes into the ripe stage.
†Only one extraction was performed.
^aSolvent amount used was 15 mL of diethyl-ether.
^bSolvent amount used was 30 mL of diethyl ether.

hormonal extraction. Soon after narcotizing the octopus, the gonad was removed, frozen in liquid nitrogen, and stored at -40°C until hormonal extraction.

Octopus maturity was confirmed both macroscopically as well as microscopically. Macroscopically in males by the creamy-white testis and Needham’s sac filled with spermatophores; and in females by the very large ovary, occupying the whole posterior half of the mantle cavity, with longitudinally striated egg yolk. Microscopically, the mature stage was verified in the gonads (i.e., testis and ovary), ovidu-

cal gland and spermatophoric complex (Fig. 1), following the procedures of Avila-Poveda et al. (2009); and staining with the modified Crossmon’s trichrome method (i.e., Groat’s hematoxylin, erythrosin B-Orange G and trypan blue, as derived from Crossmon, 1937; Gray, 1954; Gutiérrez, 1967).

Extraction of Progesterone and Testosterone from Gonads

The method of D’Aniello et al. (1996) was followed with some adjustments (i.e., timing and

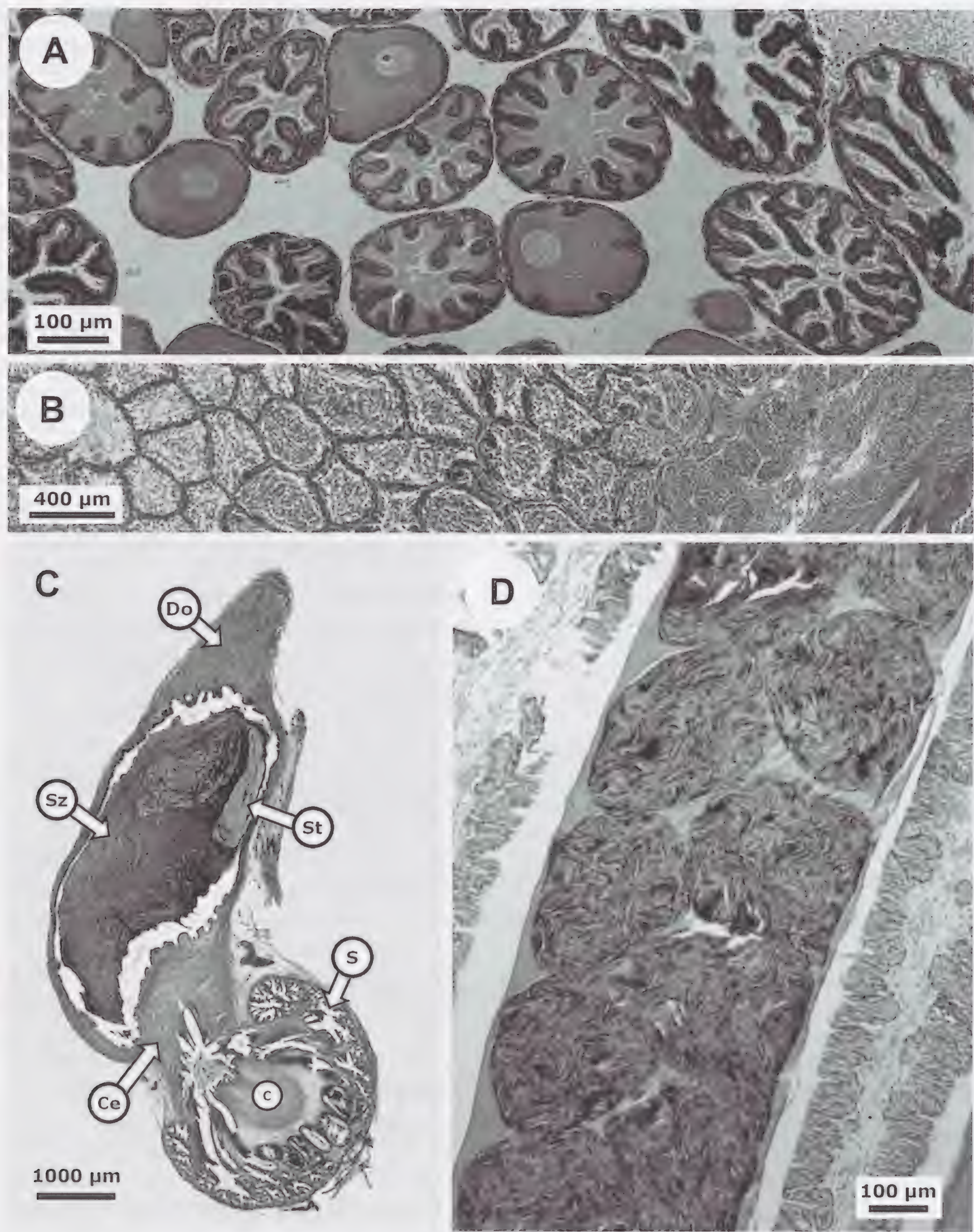


FIG. 1. Compound digital photomicrographs of histological sections of gonads and accessory glands of *Octopus maya* showing the physiological maturity and functional maturation (Arkhipkin, 1992) of the octopuses used in this study. A: Ovary at stage III, “mature”, with very large increase in oocyte diameters, primary follicle deeply embedded, and initial yolk development; B: Panoramic photomicrograph of the testis, showing Stage II (gametogenesis on the left side) to Stage III (mature on the right side and toward the accessory gland). In Stage II a mixture of spermatogonia, spermatocytes, spermatids, and spermatozoa is in the lumen of the seminiferous tubules, as well as a wide belt of spermatogonia surrounding the walls of the seminiferous tubules. In Stage III the lumen of the seminiferous tubules is filled with spermatozoa and surrounded by a narrow belt of spermatids; C: Transverse cross-section of the oviducal gland and distal oviduct of a female *Octopus maya* (373 g BW), showing the arrangement of the spermathecae (S) in several radial chambers that open into the central cavity (c) of the oviducal gland. Spermatozoa (Sz) were scarcely found within the spermathecae (S) but in a dense mass that also included a spermatophore (St) near the cervix (Ce) or neck of the oviducal gland, which corresponds to the lower narrow portion of the oviducal gland where it joins the distal oviduct (Do); D: Histological photomicrographs of the spermatophoric complex of a male *Octopus maya* (673 g BW) showing the arrangement of the spermatozoa constituting several dense masses within a spermatophore.

volume: CMFRI, 1983) which are subsequently described in detail: (1) 1 g of gonad (i.e., testis and/or ovary) was homogenized with 3 mL of distilled water for 3 min at a speed of 4000 rpm in a Tissue Homogenizing Systems Glas-Col®; (2) the homogenate was supplemented with 15 mL of diethyl-ether and vigorously mixed for 3 min (i.e., first extraction); (3) the mixture was set to rest for 45 min at 2–6°C; (4) the mixture was immersed in liquid nitrogen for 60 s and then the upper diethyl-ether phase was separated from the precipitate; (5) the extraction was repeated a second time from the precipitate obtained upon carrying out steps 2, 3 and 4; (6) the upper diethyl-ether phases were combined and evaporated in a hood with a switch-on airflow; and (7) the dry residue (i.e., gonad extract, GE) was stored at -20°C until measurement by RIA.

Preparation of Gonad Extracts for Radioimmunoassay

This procedure was developed according to Abraham (1975) and Abraham et al., (1977) and involved two additional major steps: (8) The stored GE was re-dissolved in 1000 µL methanol (M) and mixed for 3 min; (9) a 500 µL aliquot of the GE-M complex was transferred to a glass tube to which 500 µL of 0.1 M phosphate buffer solution (PBS), pH 7.0 was added and mixed for 3 min to form the GE-M-PBS complex that was used for RIA.

Standards Preparation

For both hormones, progesterone and testosterone, nine standards (in 0.1 M PBS, pH 7.0) were prepared by serial dilution and used at the following concentrations: 0.0, 0.0625, 0.125, 0.25, 0.5, 1.0, 2.0, 5.0 and 10.0 ng•mL⁻¹; covering the range of hormone levels reported from radioimmunoassay for *Octopus* spp. (Table 1).

Radioimmunoassay Procedure

All kit reagents (standards, labelled hormones, and coated tubes, including prepared gonad extracts) were at room temperature (~25°C) and mixed thoroughly to avoid foaming before use. All measurements were performed in duplicate and the duplicates were averaged into a single measurement. Solid-phase RIA for each hormone (progesterone and testosterone) was performed according to the manufacturer's

instructions that are described in detail subsequently: (1) each prepared standard and gonad extract was pipetted into the bottom of its corresponding coated-tube (50 µL for testosterone assay or 25 µL for progesterone assay); (2) 500 µL of ¹²⁵I-labeled hormone was immediately added to each coated-tube for the respective hormone assay; (3) each coated-tube was quickly vortexed; and (4) the tubes were then incubated for 60 min at 37°C in a water bath; (5) tubes were decanted, and allowed to drain until dry (around 2 min) before being read; (6) the radioactivity in each tube was quantified in a gamma-counter (Burnham-on-Crouch England) for 60 s.

Radioimmunoassay Analytical Validation

A curve-fitting (logit-log) technique was used, which applies logit (B/B₀) vs. log dose (Healy, 1972; Rodbard, 1974; Abraham et al., 1977; Govindarajulu, 2001). The reliability of the assays was validated based on their specificity, sensitivity, accuracy, precision and reproducibility.

Specificity or freedom from interference by substances other than the one to be measured was assessed by the parallelism test, assaying serially diluted ovary extracts (1:1, 1:2, 1:4 and 1:8) against the standard curve (Cekan, 1975, 1979; Hafs et al., 1977; IAEA, 1984), and plotting and testing the equality of the slopes (Zar, 2010). A lack of significant interaction between the serial dilutions and the standard curve indicates that the line slopes are similar. If the interaction is significant, then the slopes of the serially diluted sample and the standard curve are not parallel.

Practical and theoretical sensitivity of the assay was determined by calculating the smallest amount of hormone significantly different from zero at the 95% confidence limit (Abraham, 1975; Bedolla-Tovar et al., 1984; IAEA, 1984). For each hormone assay, cpm (counts per minute) of the zero standard were obtained from independent standard curves (n = 5 to 8) assayed after 6, 12, 28 and 46 days had elapsed in the half-life of ¹²⁵I:

$$\text{Practical Sensitivity} = [x - (2 * S.D.) / x] * 100$$

(IAEA, 1984)

$$\text{Theoretical Sensitivity} = 100 - [(t * C.V.) / \sqrt{n}]$$

(Bedolla-Tovar et al., 1984),

where *x*, *S.D.* and *C.V.* are the basic measurements of central tendency based on the counts per minute of the zero standard, *t* is Student's t-value tabulated to n-1 degrees of

freedom (95% confidence), and *n* is the number of zero standards measured.

Sensitivity changes throughout radioactive decay of ¹²⁵I were defined by changes in the values of the slopes of the standard curve assays on different days (Chase, 1979; Ezan et al., 1991; Alvarez-Cervera, 2002).

Assay accuracy denotes how close the results are to the true contents of unknown samples (Skelley et al., 1973; Abraham, 1975; Abraham et al., 1977; Cekan, 1975, 1979; IAEA, 1984), and was tested by adding a known quantity of standard to a GE-M-PBS complex (volume 1:2) from which all free steroids had first been removed with an active charcoal suspension of 0.6% (w/v) in PBS (Abraham et al., 1977). The accuracy of the RIA was tested by recovery experiments and was calculated as:

Recovery (%) = (Measured Value/Expected Value) x 100.

Precision of the assay was calculated by determining the intra-assay variability (CV) of duplicate measurements of the same sample in the same assay; while *Reproducibility* was determined using the inter-assay variability (CV) measuring the same sample in different assays (Skelley et al., 1973; Rodbard, 1974; Abraham, 1975; Cekan, 1975; IAEA, 1984; Ezan et al., 1991; Krotzky & Zeeh, 1995).

The intra and inter-CV were calculated as the standard deviation divided by the mean value. Generally, the inter-assay (between-assay) variance should be greater than the intra-assay (within-assay) variance; and the ratio of intra-assay to inter-assay variance provides an index of the temporal stability of the system (Rodbard, 1974).

Recovered Hormone

Previous pioneering studies with RIA in octopuses did not reveal the percentage of hormone recovered from gonads during extraction. The authors assumed that with one (Di Cosmo et al., 2001) or two extractions (Nikitina et al., 1977; D’Aniello et al., 1996; Olivares-Paz et al., 2003), the whole amount of hormone was recovered from the gonad. Then, the number of required extractions to obtain the entire hormone contained in the gonad was evaluated with a single mature ovary after making 2, 4, 6, 8 or 10 extractions, assuming that with the 10th extraction all the hormone (i.e., progesterone and testosterone) was extracted from the gonad.

Solvent Effect on Progesterone and Testosterone Extraction

Abraham (1975) indicated that the most common factor affecting the recovered concentration of hormone is the amount of solvent used during the extraction procedure; therefore, the highest quality should be used and in large amounts. Due to logistics, we used half (i.e., 15 mL of diethyl-ether) the amount of solvent indicated by D’Aniello (1996) for hormone extraction throughout this study. Even so, a complementary evaluation of the solvent effect on hormone extraction was made with a group of randomly selected samples; thus, the homogenates were extracted with 15 mL of diethyl-ether each time, but also with 30 mL of diethyl-ether in parallel (see methodology of extraction).

TABLE 2. Practical and theoretical sensitivities (ng·g⁻¹) of progesterone and testosterone assays throughout the radioactive decay of ¹²⁵I. (n = number of zero standard measured in duplicate, each derived from a standard curve).

Assay	Radioactive decay of ¹²⁵ I (days)			
	6	12	28	46
Progesterone (practical)	0.1308 (n = 6)	0.0572 (n = 6)	0.0952 (n = 4)	
Progesterone (theoretical)	0.0429 (n = 6)	0.0174 (n = 6)	0.0422 (n = 4)	
Testosterone (practical)	0.0688 (n = 5)	0.0032 (n = 4)		0.1012 (n = 8)
Testosterone (theoretical)	0.0175 (n = 5)	0.0011 (n = 4)		0.0170 (n = 8)

TABLE 3. Radioimmunoassay accuracy tested through recovery experiments (i.e., measured and expected values) for progesterone and testosterone from *Octopus maya* gonads.

Progesterone Expected Values = 3.33 ng•g ⁻¹ n = 15 samples		Testosterone Expected Values = 1.67 ng•g ⁻¹ n = 15 samples	
Measured Values	Recovery (%)	Measured Values	Recovery (%)
3.18	95.39	1.47	87.91
3.75	112.54	1.18	70.57
3.93	117.76	1.51	90.31
3.80	113.89	1.40	83.87
3.43	102.89	1.32	79.50
3.99	119.72	1.39	83.58
3.72	111.66	1.38	83.09
3.29	98.73	1.38	82.96
3.46	103.68	1.41	84.35
3.30	98.90	1.50	89.82
3.20	95.93	1.34	80.42
3.14	94.33	1.13	67.59
2.60	77.93	1.31	78.90
3.29	98.56	1.31	78.56
3.06	91.89	1.38	82.85
MEAN	102.25	MEAN	81.62
S.D.	11.22	S.D.	6.24

RESULTS

Radioimmunoassay Analytical Validation

Specificity. No significant differences were observed between the slope of the diluted sample and the standard curve for progesterone ($t_{0.05(2)10}$, $t_{cal} = 2.0943$, $P = 0.0626$) or for testosterone ($t_{0.05(2)10}$, $t_{cal} = 0.5540$, $P = 0.5917$), indicating that the serially diluted gonad extracts paralleled the standard curves of progesterone and testosterone. These results indicate linearity and specificity, i.e., that the antibody recognized the same structure immunologically in the unknown sample as in the standard curve.

Sensitivity. There were no significant differences ($P > 0.05$) between the practical and theoretical sensitivities of either hormone throughout the radioactive decay of ¹²⁵I, indicating that both determinations are consistent to show sensitivity. Sensitivities for both hormones were less than 0.1 ng•g⁻¹ (Table 2) and variations (i.e.,

values for the slopes of the standard curves) were not greater than 20% over 46 days of ¹²⁵I radioactive decay for both hormones (Fig. 2).

Accuracy. Table 3 shows the ranges of measured and expected values for progesterone and testosterone RIAs performed with two extractions. Briefly, the assay accuracy was 102.25 ± 11.22% for progesterone and 81.62 ± 6.24% for testosterone (n = 15).

Precision and Reproducibility. For both hormones, precision (intra-assay) was close to 13% and reproducibility (inter-assay) less than 17% (Table 4). The precision/reproducibility ratio was 75.39% for progesterone and 96.16% for testosterone, which indicates a good stability of the system.

Hormone Recovery

The fraction of hormone recovered with two extractions was 65% for progesterone and 83% for testosterone, but increased with a larger number of extractions (Fig. 3).

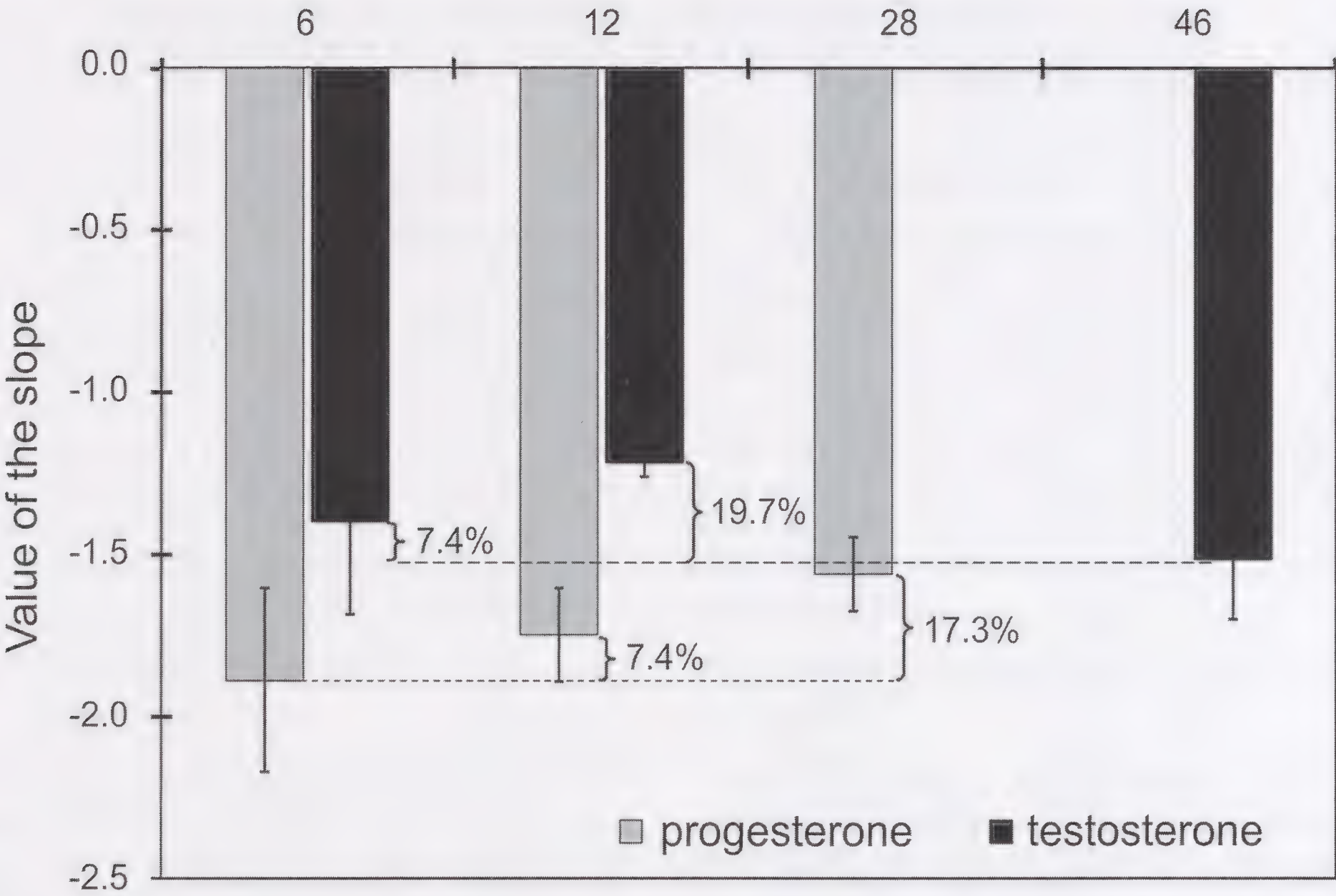


FIG. 2. Changes in progesterone and testosterone sensitivities with radioactive decay of ¹²⁵I.

TABLE 4. Intra- and inter-assay variability in tissue samples from *Octopus maya* in progesterone and testosterone RIA (sn = number of duplicate samples of the same RIA; rn = number of RIAs where the samples were assayed in duplicate; SD = standard deviation; CV = coefficient of variation).

Progesterone precision (Intra-assay variability)					Testosterone precision (Intra-assay variability)				
Sample	sn	mean (ng•g ⁻¹)	SD	CV (%)	Sample	sn	mean (ng•g ⁻¹)	SD	CV (%)
S1	2	1.64	0.10	6.03	S8	2	0.14	0.01	2.26
S2	2	1.11	0.27	24.71	S9	2	0.14	0.03	23.09
S3	2	1.48	0.13	8.89	S10	2	0.07	0.01	13.86
S4	3	1.83	0.13	6.94	S8	2	0.14	0.01	2.34
S5	2	1.50	0.27	18.22	S9	2	0.22	0.06	25.27
				12.96	S10	2	0.06	0.01	14.53
									13.56

Progesterone reproducibility (Inter-assay variability)					Testosterone reproducibility (Inter-assay variability)				
Sample	rn	mean (ng•g ⁻¹)	SD	CV (%)	Sample	rn	mean (ng•g ⁻¹)	SD	CV (%)
S4	3	2.17	0.35	16.09	S8	2	0.14	0.01	0.14
S5	3	1.78	0.40	22.49	S9	2	0.19	0.06	33.05
S6	3	0.99	0.11	11.52	S10	2	0.07	0.01	9.11
S7	2	0.64	0.12	18.65					14.10
				17.19					

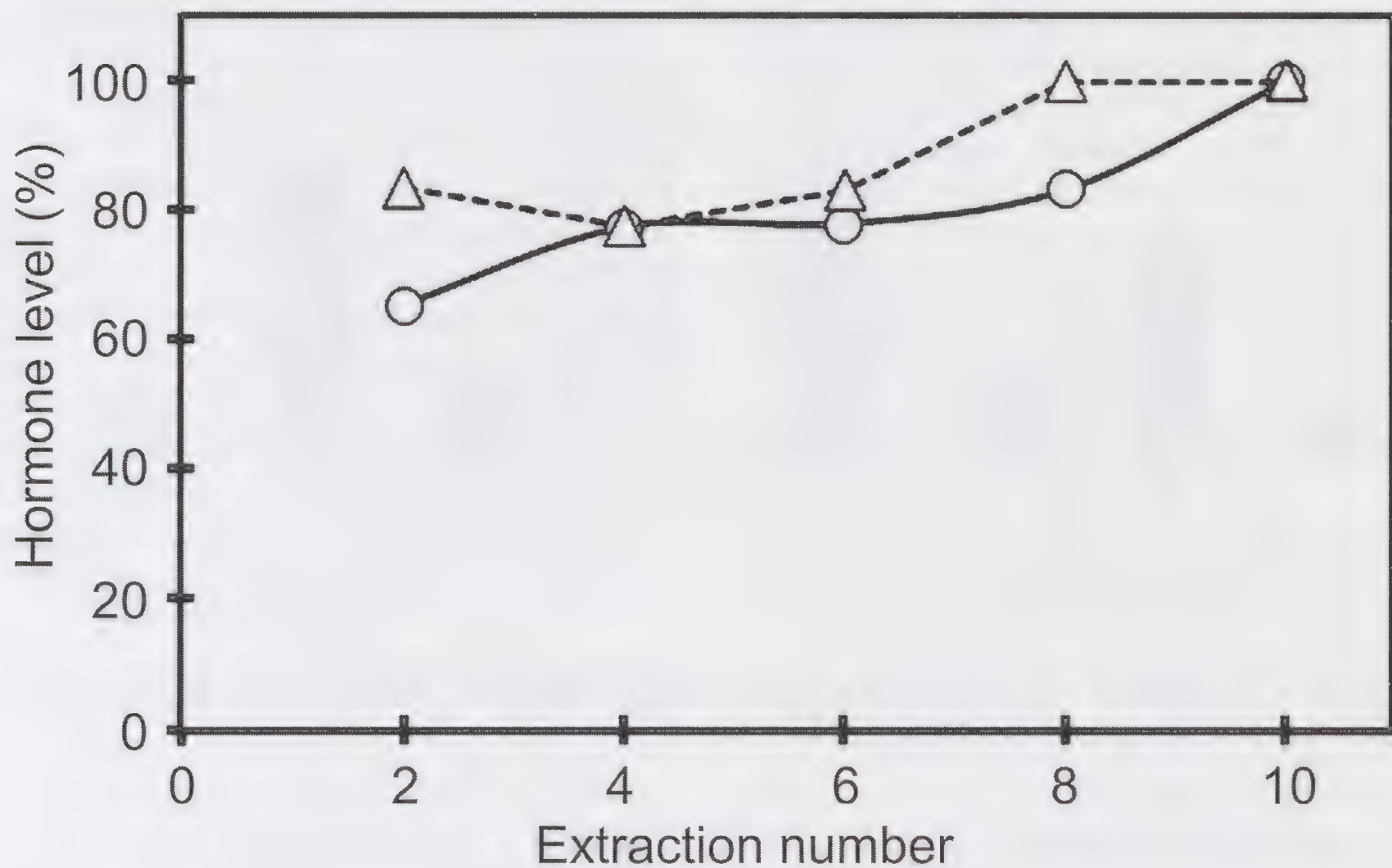


FIG. 3. Levels of recovered hormone in progesterone (○) and testosterone (Δ) radioimmunoassays according to the number of extractions made. It is assumed that with the 10th extraction all hormone in the tissue was recovered.

Solvent Effect on Progesterone and Testosterone Extraction

The concentrations of detected progesterone and testosterone following the extraction procedure with 15 mL of diethyl-ether per extraction were 4-fold lower, on average, than the levels of hormones detected following the same procedure but with 30 mL of diethyl-ether per extraction (Fig. 4, Table 5).

Progesterone and Testosterone Concentrations

Following extraction with 15 mL of diethyl-ether, the progesterone concentrations detected ranged from 0.90 to 2.22 ng•g⁻¹ in females and 0.80 to 1.60 ng•g⁻¹ in males; while, at a much lower concentration, the range of testosterone was 0.08 to 0.14 ng•g⁻¹ in females and 0.05 to 0.22 ng•g⁻¹ in males (Table 5).

TABLE 5. Amount of solvent used during the extraction procedure for RIA and the steroid level (i.e., progesterone and testosterone) reported. Authors within each source are ordered according to year of publication.

Tissue (g)	Solvent	Solvent amount	Extraction numbers	Progesterone (ng•g ⁻¹)	Testosterone (ng•g ⁻¹)	Sources
ovary, 5	acetone	No data	two	8.8	No data	(Nikitina et al., 1977)
testis, 1	diethyl-ether	30 mL	two	~3.3	~5.3	(D'Aniello et al., 1996)
ovary, --	methanol-water	70–30%	one	~0.020 to 0.200	No data	(Di Cosmo et al., 2001)
testis, --	diethyl-ether	5 volumes	two	~3.5 to 8.2	~5.0 to 9.5	(Olivares-Paz et al., 2003)
ovary, 1	diethyl-ether	15 mL	two	0.90 to 2.22	0.08 to 0.14	This study
ovary, 1	diethyl-ether	30 mL	two	4.42 to 10.11	0.28 to 0.56	This study
testis, 1	diethyl-ether	15 mL	two	0.80 to 1.60	0.05 to 0.22	This study
testis, 1	diethyl-ether	30 mL	two	3.96 to 6.57	0.30 to 0.67	This study

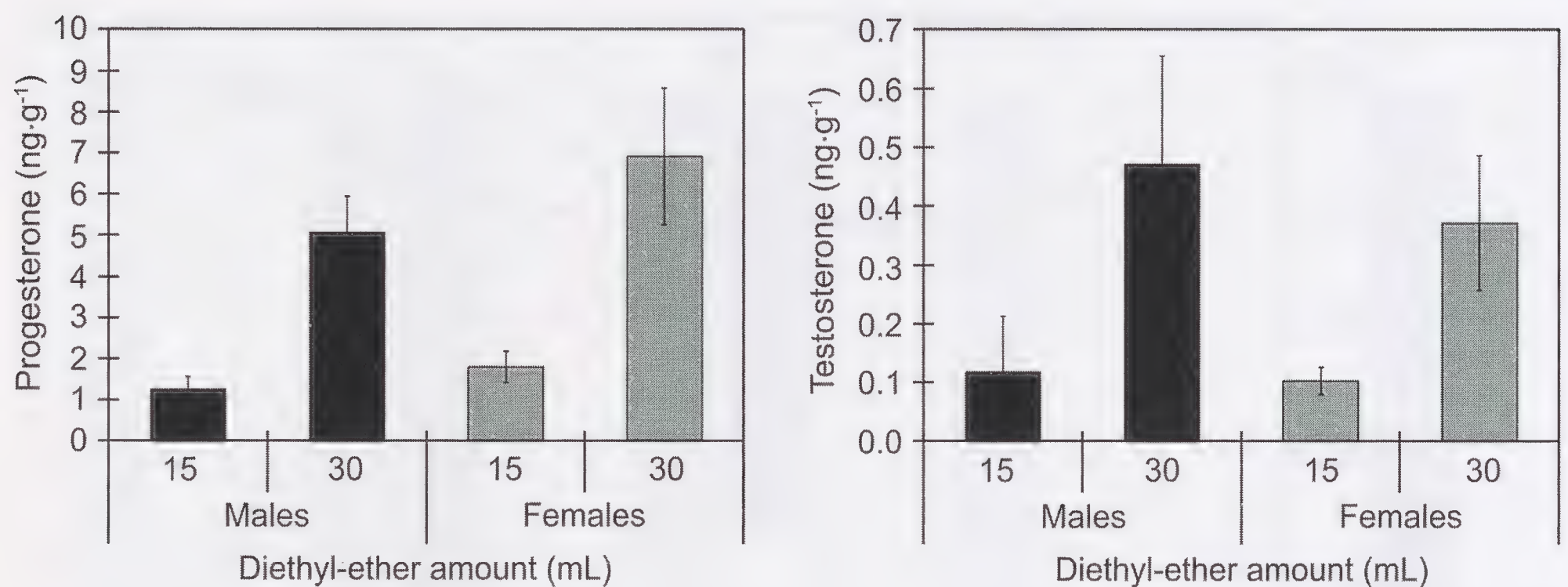


FIG. 4. Effect of the amount of diethyl-ether on the level of detected progesterone and testosterone for twenty-two samples processed by radioimmunoassay.

DISCUSSION

Commercial RIA kits offer 17β -estradiol, testosterone, and progesterone specific antibodies that identify the common structures present in humans. Here in the present study it was demonstrated that the required acceptance criteria for validated RIA parameters were fulfilled. This means that with adequate extraction procedures it is possible to quantify “vertebrate-type” sex steroid hormones (e.g., testosterone and progesterone) in octopus gonad extracts using components from commercially available RIA kits (i.e., ^{125}I -labeled hormone and coated tubes). This approach represents savings in both time and money, by eliminating time lost in screening animals potentially producing steroid antisera and the costs of maintaining them (Montes-Pérez & Pech-Martínez, 1998). It is widely acknowledged that sex steroid hormones are ubiquitous in all the animal kingdom and that they perhaps share the same common molecular formula with their vertebrate counterparts, although possibly with different structural homologies and functional analogies in distinct species (Sandor, 1980; Finch & Rose, 1995; Stoka, 1999; Lafont, 2000; Lafont & Mathieu, 2007).

Sex steroid hormones (e.g., progesterone and testosterone) in tissues of invertebrates are difficult to estimate and compare because of variations among species, organs, extraction procedures, and quantification methods (Lafont & Mathieu, 2007). Nevertheless, to our knowledge our study shows for the first time in *Octopus maya* a reliable radioimmunoassay

technique, fulfilling all recommended acceptability criteria (i.e., specificity, sensitivity, accuracy, precision, and reproducibility) (Skelley et al., 1973; Rodbard, 1974; Abraham, 1975; Cekan, 1975; Abraham et al., 1977; Hafs et al., 1977; Cekan, 1979; Chase, 1979; Bedolla-Tovar et al., 1984; IAEA, 1984; Ezan et al., 1991; Krotzky & Zehe, 1995; Alvarez-Cervera, 2002). Unfortunately, radioimmunoassay techniques used to quantify progesterone and testosterone in other octopuses such as *Eledone cirrhosa* (Nikitina et al., 1977; Young, 1992), *Octopus mimus* (Olivares-Paz et al., 2003), and *Octopus vulgaris* (D’Aniello et al., 1996; Di Cosmo et al., 2001) were not clear enough in their extraction procedure to be followed as a standardized technique. In other hands, some of these RIAs were developed using tritium (^3H) as tracer (D’Aniello et al., 1996; Di Cosmo et al., 2001). ^3H has a relatively low specific activity (1.07 PBq/mol) with respect to ^{125}I , but it is a radiation hazard when inhaled, ingested via food or water, or absorbed through the skin. Meanwhile, ^{125}I is preferred due to its higher specific activity (80.26 PBq/mol) and shorter half-life and, as a direct result, lower radioactivity with reduced hazards (AB, 2002).

On the other hand, only some acceptability criteria have previously been evaluated in octopus RIAs. The smallest amounts of hormones significantly different from zero detected (i.e., sensitivity) were 0.007 ng·g⁻¹ progesterone in *Octopus vulgaris* (Di Cosmo et al., 2001) and 0.005 ng·g⁻¹ in *Octopus mimus* (Olivares et al., 2003); while testosterone sensitivity has only been reported by Olivares et al. (2003) as

around $0.003 \text{ ng}\cdot\text{g}^{-1}$. In our study, sensitivities were $0.06 \text{ ng}\cdot\text{g}^{-1}$ for progesterone and $0.03 \text{ ng}\cdot\text{g}^{-1}$ for testosterone. But, on the basis of our results, it was evident that the changes in sensitivity (defined by the value of the slope of the standard curves) for both progesterone and testosterone did not vary more than 20% while the half-life of the ^{125}I elapsed. Moreover, all standard curves showed a strong positive correlation ($r = 0.99$) as demonstrated by the curve-fitting "logit-log" method; suggesting that the sensitivity of both RIAs is, therefore, relatively stable and acceptable throughout the entire ^{125}I half-life, and thus can be used with confidence during this period.

Our results thus indicate that precision and reproducibility were satisfactory in both hormone radioimmunoassays, where the value of %CV should not exceed $\pm 20\%$ (Abraham et al., 1977; Krotzky & Zeeh, 1995) or where the temporal stability of the system (i.e., ratio of "within-assay" to "between-assay" variance) must fall over 70% (Rodbard, 1974). Similarly, some coefficients of variation have been reported for hormone RIAs in other octopuses, such as *Octopus mimus* (progesterone, 5% average intra-assay; testosterone, 4% average intra-assay, Olivares et al., 2003) and *Octopus vulgaris* (progesterone, 8% intra-assay and 10% inter-assay, Di Cosmo et al., 2001). According to Rodbard (1974), Abraham (1975), Cekan (1975, 1979), Hafs et al. (1977), Bedoya-Tovar et al. (1984), IAEA (1984) and Ezan (1991) precision and reproducibility are the most essential components for hormone RIAs, and must be updated after each assay and when a process of sequential decision-making is used.

The range of *Octopus maya* gonad progesterone and testosterone concentrations quantified in this study encompasses the range found in other octopuses, such as *Eledone cirrhosa* (Nikitina et al., 1977; Young, 1992), *Octopus mimus* (Olivares-Paz et al., 2003), and *Octopus vulgaris* (D'Aniello et al., 1996; Di Cosmo et al., 2001). In the literature, there is little information on the percentage of recovered hormone. In fact, the great variability in progesterone and testosterone concentrations reported (Table 1) could be related to the solvent used to extract the hormones, the amount of solvent, and the number of extractions that were applied to recover the hormones from tissue (Abraham, 1975; Abraham et al., 1977). For example, Nikitina et al. (1977) performed two extractions

with acetone, D'Aniello et al. (1996), Olivares-Paz et al. (2003) performed two extractions with diethyl-ether, and Di Cosmo et al. (2001) performed one extraction with methanol. In our study it was observed that the amount of solvent used during extraction is a determining factor in the recovered concentration of hormone, since increasing the solvent amount (i.e., 15 to 30 mL of diethyl-ether per each extraction) also increased the amount of recovered hormone. Abraham (1975) and Abraham et al. (1977) reported this same effect.

The requirement for more than one extraction could be related to the lipid matrix in the tissue that commonly interferes with radioimmunoassay performance (Abraham, 1975; Abraham et al., 1977; Hafs et al., 1977; Rash et al., 1980). Octopus ovaries accumulate egg-yolk vitellins (i.e., the major yolk lipoproteins that serve as the nutritive material for development of the embryo and early larva) during maturation (O'Dor & Wells, 1973; Gnap, 1987; Pollero & Iribarne, 1988; Boyle & Chevis, 1992; Rosa et al., 2004; Siero et al., 2006, among many others); therefore, this condition should be taken into account, particularly in females, when hormones are being extracted. This problem could be solved by using more than two extractions, as was observed here.

An extremely important factor in determining the magnitude of the estimated concentration of hormones in a gonad extract is the nature of the method by which the hormonal concentrations are measured. It has been reported that precise and accurate assays such as RIA do not show identical results with other extremely sensitive, accurate and precise analytical methods such as HPLC-EIA (Van de Wiel & Koops, 1986) and LC-MS/MS (Gust et al., 2010). Several authors (Abraham, 1975; Cekan, 1975, 1979; IAEA, 1984; Srikandakumar et al., 1986; Ezan et al., 1991; Chard, 1995; Montes-Pérez, 1995; Alvarez-Cervera, 2002, among many others) have indicated that the amount of quantified hormone is highly variable according to: (1) immunoassay type (liquid-phase or solid-phase); (2) type of label on the tracer (Iodine or Tritium); (3) sample treatment method (direct or indirect); (4) antibody specificity; and (5) type of standards (commercial or elaborated).

Therefore, the reported concentrations of hormones as determined by RIA correspond to relative magnitudes, which are reliable according to the used method. In any case, the

meaning of the considered value is not only based on the criteria which the method was validates, but is also based on the consistency and validity of the method itself and the congruence of these values with the biological process being studied, rather than by “confirmative proofs” with other analytical methods (Montes-Pérez, 1995; Escrig-Sos et al., 2006; Gust et al., 2010).

In summary, it can be concluded that the developed and validated RIAs meet the requirements for the quantification of progesterone and testosterone in *Octopus* gonad extracts and show that it is possible to use ^{125}I -labeled hormone and plastic coated-tubes from commercial RIA kits. Therefore it is possible to use the procedures of these RIAs to analyse variations in hormone levels associated with gonad development stages and the reproductive cycle of octopuses, enabling investigations of their possible functions.

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SPECIES CATALOGUE OF ORTHALICOIDEA IN ARGENTINA (GASTROPODA: STYLOMMATOPHORA)

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ABSTRACT

We provide here a catalogue of all available species nomina of Orthalicoidea occurring in Argentina. Ongoing taxonomic revisions on the genera *Bostryx* Troschel, 1847, *Clessinia* Doering, 1874, *Pilsbrylia* Hylton Scott, 1952, and *Spixia* Pilsbry & Vanatta, 1898, highlighted the necessity of an updated catalogue for the region. A total of 101 orthalicoidean species classified into four families, Bothriembryontidae, Bulimulidae, Odontostomidae and Simpulopsidae are present in Argentina. The catalogue provided here is based on examination of primary literature, available revisions and monographs, comparative studies within and among species and revision of museum data, including most type specimens. Additional collection of specimens in various localities of the country was carried out for more than a decade to be able to accurately state distributional information on the species treated. Nomenclatural details are provided for all nominal species. Name-bearing types were located for 86 species-group taxa, and six lectotypes were designated for the stabilization of the taxonomy. We propose the following nine new combinations: *Bulimulus fourmiersi* (d'Orbigny, 1835), *Clessinia cordovana* (Pfeiffer, 1855), *Drymaeus flossdorfi* (Holmberg, 1909), *Cyclodontina* (*Ventania*) *avellanadae* (Doering, 1881), *Simpulopsis* (*Eudioptus*) *eudioptus* (Ihering in Pilsbry, 1897), *Spixia champaquiana* (Doering, 1875), *S. charpentieri* (Grateloup in Pfeiffer, 1850), *S. minor* (d'Orbigny, 1837) and *S. parodizi* (Hylton Scott, 1951). The following four new synonymies are proposed: *Bostryx sophieae* Breure, 1979, with *Bulimus cordillerae* (Strobel, 1874) (current name *Bostryx cordillerae*); *Cyclodontina* (*Clessinia*) *gracilis* Hylton Scott, 1956, with *Bulimus cordovanus* Pfeiffer, 1855 (current name *Clessinia cordovana*); *Spixia estherae* Fernández, 1971, with *Odontostomus* (*Spixia*) *costellifer* Hass, 1936 (current name *Spixia costellifer*); *Kuschelenia simulans* Hylton Scott, 1951, with *Helix tupacii* d'Orbigny, 1835 (current name *Scutalus tupacii*). *Bulimulus sporadicus gracilis* Hylton Scott, 1948, is changed from subspecific to specific status.

Key words: South America, Pulmonata, Stylommatophora, available nomina.

INTRODUCTION

Orthalicoidea is a large taxon and a major component of the land gastropod fauna in South American countries. This monophyletic superfamily is distributed in Melanesia, New Zealand, Australia, Africa, South America, the West Indies and Central America north to southern United States (Breure et al., 2010; Breure & Romero, 2012).

The taxonomic composition, as well as the status of their components, is a subject of debate to present days. The group was

originally divided into five subfamilies by Pilsbry (in various works between 1895 and 1902) on the bases of anatomical characters. Zilch (1959–1960) divided Bulimulacea (= Orthalicoidea) into six families that also included Cerionidae and Urocoptidae, now classified into a different superfamily. Later, Breure (1979) on the grounds of anatomical information, considered Zilch families as subfamilies of Bulimulidae. Breure et al. (2010) using DNA sequences of 22 taxa spanning all the Orthalicoidea, explored the phylogenetic relationships within the superfamily and concluded that the

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clades (A: Bulimulidae, B: Odontostomidae, C: Bothriembryontidea, D1: Orthalicinidae, D2: Amphibulimidae) obtained in their analysis should be given familiar status. Breure (2011) also gave family rank to Simpulopsidae Schileyko, 1999, which is now classified together with the other five families into Orthalicoidea. Recently, Breure & Romero (2012) proposed a new molecular phylogenetic hypothesis of Orthalicoidea with strong support for its monophyly as well as for such clades as Bulimulidae, Bothriembryontidae, Amphibulimidae, Simpulopsidae and Orthalicidae. Other groups such as Odontostomidae and Megaspiridae showed low support and appeared in their analysis as paraphyletic. Previous published catalogues and check lists reporting on this superfamily in Ecuador (Breure & Borrero, 2008), Colombia (Linares & Vera, 2011), Brazil (Campos Salgado & Santos Cohelo, 2003; Simone, 2006) and Peru (Ramirez et al., 2003) showed that this superfamily is one of the most diverse and species rich of all Neotropical land gastropods. However, vast areas in South America still remain unknown concerning to such basic questions as which or where species occur.

In Argentina, Orthalicoidea represent the richest group of land snails and is widely distributed in almost all the different ecoregions, with the exception of southern areas and Antarctica. Only four out of the seven families composing Orthalicoidea (sensu Breure & Romero, 2012), Bothriembryontidae, Bulimulidae, Odontostomidae and Simpulopsidae, inhabit in Argentina, being Odontostomidae and Bulimulidae the richest and phylogenetically diverse.

Land gastropods in Argentina were studied during the nineteenth century and most species were described since then, but generic revisions are still scarce. Alcide d'Orbigny (1834–1847, 1835) was one of the most prolific researchers and the author of 33 land gastropod species of Argentina. In Orthalicoidea, most of *Drymaeus* Albers, 1850, species were described by him. Adolfo Doering, who emigrated from Germany to Argentina to work at the Academy of Sciences of Cordoba, had a great influence in the development of malacology in Argentina in general and especially in the study of odontostomids land snails. Doering is the author of around 30 bulimulid and odontostomid species. A colleague and contemporary researcher of Doering, Eduardo L. Holmberg, also described *Bulimulus* and *Bostryx* species from Argentina. Later, in the mid twentieth century such other researchers as M. I. Hylton Scott, J. J. Parodiz

and W. Weyrauch gave impetus to pulmonate gastropods research in Argentina describing numerous new species from different areas and reviewing several genera. Barbosa et al. (2008) provided a complete list of species published by W. Weyrauch.

Previous species catalogues of Argentinean land gastropods were prepared by Parodiz (1957a, b) reporting the existence of 97 taxa, and Fernández (1973a, b) recording 114 orthalicoidean species in the country. However, many taxonomic changes, especially concerning the status of genera and families, as well as their species composition have changed since then. There is an urgent need to accurately identify neotropical land snail species for biodiversity, conservation, phylogenetic and phylogeographic studies. Taxonomic knowledge remains essential to credible biological research and is made urgent by the biodiversity crisis (Wheeler, 1995, 2004). As pointed by Dubois (2010), the nomenclatural rules should be applied to correctly transmit and communicate about the living organisms. Failure to use correct names and spellings may cause ambiguities in communication and therefore increase our current taxonomic crisis (Wheeler, 1995; Dubois, 2010).

The object of this research is to provide an updated catalogue of the orthalicoidean species recorded in Argentina establishing their current classification and distribution.

MATERIAL AND METHODS

The material in this catalogue is housed in the main Argentinean malacological collections at public museums as well as in European and North American museums. The systematic in this study follows the arrangement proposed by Breure et al. (2010) and Breure (2011). Within each family, genera and species are arranged in alphabetical order.

The type locality for each species is given in quotation marks as it is stated in the original publication and in the same language used by the author. In some cases, further detail was added to clarify the geographic location and to give more precision to each type locality. This procedure is used because there were cases in which authors repeated the type locality as it is mentioned in previous publications. In some of these cases, the type locality is different from what was stated in the original species description.

Year of publication of species originally described by A. Doering are referred exactly as they were printed in each volume of the *Boletín de la Academia Nacional de Ciencias de Córdoba* or in *Periodico Zoológico*. Following the nominal year of publication, we have included in brackets the year cited as probable actual date of publication, in most cases in concordance with Pizá & Cazzaniga (2003, 2006, 2010, 2012).

The type material of each species have been search and consulted in museums where the author of each species worked. Although type material was found in most cases, in some other cases name-bearing types have been lost, especially concerning species described by A. Doering. In each case, the location of the type material is recorded and additional information is included in the Remarks when types were not found. We also added, when possible, the location of the types of species previously synonymized with the valid species name (in square brackets). Number of paratypes or paralectotypes specimens, in cases when possible, is included in parentheses after acronym and collection number.

Nomenclatural changes proposed in the present catalogue are shown in square brackets, previous changes established by other authors are shown in parentheses. In the species synonymy list, when the same author has several works one after another, the author's name in the first reference is followed by the successive dates without repeating author's name.

Lectotype fixation was done from syntypes to become the unique bearer of the name of a nominal species-group taxon in order to preserve stability of nomenclature according to Art. 74.1 and Art. 74-7 of the *International Code of Zoological Nomenclature* (ICZN). In each case, lectotypification was done with care and only under special conditions, following Köhler (2007). A specimen was selected to be a lectotype under the following criteria: (1) the measurements and figures given in the original description matches the selected specimen, (2) the locality was the same as that in the original species description, (3) no other type was known to exist, (4) the lot from which the relevant specimen was selected was part of the type series. Historical information was gathered in each malacological collection and author handwriting photographed and compared with lot labels. Following this procedure, the current lectotype designations are considered to contribute to a stable taxonomy. Shell mea-

surements (H, total shell length, DM, major diameter, Hap, total length of the aperture, Dap, diameter of the aperture) of new designated lectotypes and paralectotypes are provided. In cases in which no holotype or syntypes was fixed for a certain species and the taxon was based on more than one specimen, Recommendation 73F of the ICZN was followed, and the specimens were considered as syntypes rather than assuming a holotype. Synonymies proposed for the names listed below are based on the study of types, authoritatively identified specimens and original species descriptions and figures.

The distribution of genera is indicated at the level of the country. Current species distribution in Argentina is detailed with the province in italics and localities where they were collected, arranged in alphabetical order. Collection numbers are in parentheses. When only the province (Prov.) with no specific locality of occurrence is indicated on the label of the lot, the number of the lot follows directly after the province. When a question mark (?) is included after the country or province of occurrence, this means that there is doubt concerning this geographic location for the species in question. When "*" preceeds the name of a species in the catalogue, this means that this species is only mentioned to be present in the literature, but no voucher specimen is present at any collection, and the species was not found in recent field work in any locality in Argentina.

Most material deposited at the Instituto Fundación Miguel Lillo corresponds to specimens collected within at least the last ten years, meaning that current occurrence of the species in the mentioned localities was checked in the field. Total area distribution of each species is then determined on the bases of museum records and recent field work. In cases when occurrences of a species in other areas were obtained from literature citations, but for which there were no voucher specimens in museums, these localities have been noted in the Remarks for each species. Figures 1 and 2 illustrate type species of Orthalicoidea housed in Argentinean museums.

Museum and Collection Acronyms

The location of the name-bearing types is cited for each nominal species, when possible. The collections housing these name-bearing types are listed along with the acronyms used in the text.

ANC	Academia Nacional de Ciencias, Cordoba, Argentina	RESULTS
ANSP	Academy of Natural Sciences, Philadelphia, Pennsylvania, USA	Catalogue of Taxa, Synonymies and Type Data
CMNH	Carnegie Museum of Natural History, Pittsburgh, Pennsylvania, USA	Class Gastropoda Cuvier, 1798
FLMNH	Florida Museum of Natural History, Gainesville, Florida, USA	Subclass Heterobranchia Haszprunar, 1985
FMNH	Field Museum of Natural History, Chicago, Illinois, USA	Order Pulmonata Cuvier, 1817
IFML	Instituto-Fundación Miguel Lillo, Tucuman, Argentina	Suborder Stylommatophora Schmidt, 1856
MACN	Museo Argentino de Ciencias Naturales “Bernardino Rivadavia”, Buenos Aires, Argentina	Superfamily Orthalicoidea Albers, 1860
MACN-In	Invertebrate Collection	Family Bothriembryontidae Iredale, 1937
MACN-Pi	Paleoinvertebrate Collection	Genus <i>Discoleus</i> Breure, 1978
MCNL	Museo de Ciencias Naturales José Lorca, Mendoza, Argentina	Type Species: <i>Eudioptus aguirrei</i> Doering, 1884 [original designation].
MCZ	Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts, USA	Geographic Distribution: Argentina.
MLP	Museo de Ciencias Naturales de La Plata, Buenos Aires, Argentina	<i>Discoleus aguirrei</i> (Doering, 1884) Fig. 1A
NHMUK	The Natural History Museum, London, UK	<i>Eudioptus aguirrei</i> Doering, 1884: 112, pl. 2, fig. 2; – Breure, 1974b: 111.
MNHN	Muséum National d’Histoire Naturelle, Paris, France	<i>Eudioptus mendozanus bonaërensis</i> Doering, 1884: 111, pl. 2, fig. 1.
MZSP	Museo de Zoologia San Paulo, San Paulo, Brazil	<i>Eudioptus mendozanus Azulense</i> Doering, 1884: 111.
RMNH	Nationaal Natuurhistorisch Museum / Naturalis, Leiden, The Netherlands	<i>Bulimulus (Bulimulus) ventanensis</i> Pilsbry, 1896: 189, pl. 1, fig. 8.
RBINS	Royal Belgian Institute of Sciences, Brussels, Belgic	
SMF	Natur-Museum Senckenberg, Frankfurt am Main, Germany	
USNM	National Museum of Natural History, Washington, D.C., USA	
ZMB	Museum für Naturkunde, Humboldt Universität, Berlin, Germany	
ZSM	Zoologische Staatssammlung München, Germany	

→

FIG. 1. Type material of Bulimulidae species housed in Argentinean museums. A: *Discoleus aguirrei* (Doering, 1884) (lectotype ANC s/n); B: *Bostryx birabenorum* Weyrauch, 1965 (holotype IFML 985 a); C: *Bostryx catamarcanus* (Parodiz, 1956) (paratype MLP 11440); D: *Bostryx costellatus* (Hylton Scott, 1971) (holotype MACN-In 4221-1); E: *Bostryx famatinus* (Doering, 1879) (lectotype MACN-In 3233); F: *Bostryx pastorei* (Holmberg, 1912) (lectotype MACN-In 1517); G: *Bostryx reedi* (Parodiz, 1947) (holotype MACN-In 10001); H: *Bostryx rudisculptus* (Parodiz, 1956) (holotype MACN-In 380); I: *Bostryx stelzneri scaber* (Parodiz, 1948) (paratype MACN-In 4221); J: *Bostryx strobeli* (Parodiz, 1956) (holotype MACN-In 9916); K: *Bostryx willinki* Weyrauch, 1964 (holotype IFML 121a); L: *Bulimulus elatior* Hylton Scott, 1952 (paratypes IFML 735); M: *Bulimulus prosopidis* Holmberg, 1912 (lectotype MACN-In 1309); N: *Drymaeus flossdorfi* (Holmberg, 1909) (lectotype MACN-In 1352); O: *Drymaeus lynchi* Parodiz 1946 (holotype MACN-In 1344); P: *Naesiotus willinki* Breure, 1978 (holotype IFML 1263a); Q: *Kuschelenia simulans* Hylton Scott, 1951 (holotype MLP 11219). Photographs of *B. costellatus*, *B. famatinus*, *B. rudisculptus*, *B. strobeli* and *B. reedi* were provided by A. Tablado. Scale bar = 1cm.



Bulimulus (Bulimulus) aguirrei – Pilsbry, 1898: 320.

Bulimulus (Mormus) aguirrei – Holmberg, 1912b: 149.

Bulimulus (Lissoacme) aguirrei – Parodiz, 1944: 4.

Peronaeus (Lissoacme) aguirrei – Parodiz, 1946b: 315; 1947: 24; 1957b: 23; Fernández, 1969: 119, figs. 1–4; 1973a: 89.

Peronaeus (Lissoacme) azulensis – Parodiz, 1957b: 23; Fernández, 1970: 65, figs. 1–4.

Discoleus aguirrei – Breure, 1978: 198, figs. 335–340, pl. 22, figs. 7, 8; Breure, 1979: 91; Miquel, 1998: 179, figs. 3, 12, 19; Richardson, 1995: 94.

Discoleus azulensis – Breure, 1978: 198, fig. 341.

Discoleus azulensis mendozanus – Breure, 1979: 91.

Type Locality: “Cerro Claraz, Cerro de la Piedra Movediza, Sierra Tolosa”. The type locality is located in Tandil, Buenos Aires Prov.

Type Material: lectotype ANC s/n; paralectotypes ANC s/n (2) [present designation]. Lectotype: H = 23.3, DM = 11.3, Hap = 13.3, Dap = 8.1; paralectotype 1: H = 19.6, DM = 10.2, Hap = 11.7, Dap = 7.4; paralectotype 2: H = 23.5, DM = 12.0, Hap = 13.6, Dap = 9.2.

Distribution: Argentina; *Buenos Aires Prov.*, Abra del Pantanosa (MACN-In 26624), Azul (MACN-In 13058), Sierra de Cura Malál (MACN-In s/n), Tandil (MLP 7990, IFML 15657). *La Pampa Prov.* (MLP 7987), Lihuel Calel Dept. (MACN-In 6184; MLP s/n, IFML 15658).

Remarks

Material from the type series of *Eudioptus aguirrei* Doering, 1884, was found at the Zoological Museum of the Academia Nacional de Ciencias in Cordoba, Argentina, where Doering worked from 1872. This material is composed of three dry shells matching with the original description, type locality, similar dimensions and shell measurements provided by Doering (1884). The original label of this material is handwritten by Adolf Doering. Eduardo Holmberg gave the mollusks material gathered during his travels to Sierra de la Ventana (Holmberg, 1884) to Doering for identification, it was collected in Cerro Claraz during February 1882 by Eduardo Aguirre, an engineering working with Holmberg while they were prospecting the area of Sierra de la Ventana. At that time, Holmberg, as he explained himself, concentrated on the identification of insects and spiders of the area.

Discoleus ameghinoi
(Ihering, 1908)

Bulimulus ameghinoi Ihering, 1908: 430, fig. 1.
Bulimulus sporadicus pampa Ihering, 1914: 70.

Bulimulus (Lissoacme) ameghinoi – Parodiz, 1944: 1.

Peronaeus (Lissoacme) ameghinoi – Parodiz, 1946b: 315, pl. 1, fig. 2, pl. 2, fig. 2; 1947: 23; 1957b: 24; Fernández, 1969: 120, figs. 5–8; 1973a: 90.

Peronaeus (Lissoacme) ameghinoi madrynnensis – Parodiz, 1944: 3; 1947: 24; 1957b: 24; Fernández, 1973a: 91.

Discoleus ameghinoi – Breure, 1979: 91; – Miquel, 1998: 181, figs. 1, 2, 4, 17, 19.

Type Locality: “étage bonaëreén du ruisseau Chapalmalán”. According to Miquel (1998) the type locality of *D. ameghinoi* is Banks of the river Chapadmalal, Gral. Pueyrredon, Buenos Aires, Argentina.

Type Material: holotype MACN-Pi 562a.
Peronaeus ameghinoi madrynnensis holotype MACN-In 25878.

Distribution: Argentina, *Buenos Aires Prov.* (MACN-In 562, MACN-In 25878*), Tandil (MACN-In 16334, MACN-In 15361, MACN-In 6184). *Chubut Prov.*, Viedma Dept., Doradillo (MACN-In 25878, MACN-In 25878-1), Península Valdez (MACN-In s/n). *Río Negro Prov.*, San Antonio Dept., Fuerte Argentino (MACN-In 19555), Las Grutas (IFML 15659 A), San Antonio (MACN-In 31124, MLP 3109, MLP 7998, MLP 7999, MLP 7993, MLP 7994), Madryn (MLP 7996). *Santa Cruz Prov.*, Deseado Dept., Bahía de Sanguineto (MACN-In 18445).

Genus *Plectostylus*
Beck, 1837

Type Species: *Bulimus peruvianus* Bruguière [subsequent designation by Gray, 1847].

Geographic Distribution: Argentina, Chile.

Plectostylus mariae
Brooks, 1936

Plectostylus mariae Brooks, 1936: 124; – Parodiz, 1957b: 24; Hylton Scott, 1963: 396; Stuardo & Valdovinos, 1985: 57; Miquel, 1998: 183, figs. 5–7, 13, 16, 19.

Plectostylus vagabondiae Brooks, 1936: 125;
– Valdovinos & Stuardo, 1988: 138, figs. 2,
30–32, 41, 49, 55, 59, 70, 74.
Plectostylus argentinensis – Parodiz, 1951: 334.

Type Locality: “San Esteban, Chile”.

Type Material: lectotype CMNH 6228184.
Plectostylus argentinensis holotype MACN-In
6514; paratype MACN-In 6515.

Distribution: Argentina, *Chubut Prov.*, Futaleufú
Dept., Lake Menendez (MACN-In 30607).
Neuquen Prov., Lacar Dept., Lake Lacar (MACN-
In 32845, MACN-In 26345), San Martín de los
Andes (MACN-In 6514, MACN-In 6515).

Familia Bulimulidae
Tryon, 1867

Genus *Bostryx*
Troschel, 1847

Type Species: *Bulimulus (Bostryx) solutus* Tro-
schel, 1847 [original designation by monotypy].

Geographic Distribution: Argentina, Bolivia,
Chile, Perú, Colombia, Ecuador, Paraguay.

Bostryx birabenorum
Weyrauch, 1965
Fig. 1B

Bostryx (Lissoacme) birabenorum Weyrauch,
1965: 71, figs. 1–3; – Fernández, 1973a: 99;
Neubert & Janssen, 2004: 202.
Bostryx birabenorum – Miquel, 1993: 158, pl.
1, figs. 1, 2.

Type Locality: “Argentina, Provincia de Tucuman,
El Infiernillo cerca de Tafi del Valle, 3042 m”.

Type Material: holotype IFML 985 a; para-
types IFML 985 b (7), MLP 11620 (3), SMF
164112 (2).

Distribution: Argentina, *Tucuman Prov.*, Tafi
del Valle Dept., El Infiernillo (IFML 985, IFML
14924, MLP 11620), Valles Calchaquies
(MACN-In 23041, MLP 8122).

Bostryx catamarcanus
(Parodiz, 1956)
Fig. 1C

Bulimulus (Scansicochlea) catamarcanus
Parodiz, 1956b: 77, fig. 6; – Fernández, 1973a: 82.

Bostryx (Lissoacme) catamarcanus – Weyrauch,
1965: 73.

Bostryx catamarcanus – Breure, 1979: 52;
Miquel, 1993: 159, pl. 1 fig. 5.

Type Locality: “Concepción, provincia de Ca-
tamarca”. The locality is into the Capayán
Dept, Catamarca.

Type Material: holotype MACN-In 17778;
paratypes MACN-In 17778-1 (78), MLP
11440 (2).

Distribution: Argentina, *Catamarca Prov.*, Belén
Dept., Sierra de Belén (MLP 8061); Capayán
Dept., Concepción (MACN-In 17778, MACN-
In 17778-1, MLP 11440).

Bostryx cordillerae
(Strobel, 1874)

Bulimulus (Mesembinus) cordillerae Strobel,
1874: 22, pl. 1, fig. 3.

Bulimus (Mesembinus) Cordillera – Doering,
1875a [1877]: 340 [incorrect subsequent
spelling].

Bulimulus (Bulimulus) cordillerae – Doering,
1879: 72.

Bulimulus (Bulimulus) monticola Doering,
1879: 69, *non* Philippi, 1869 (secondary
homonymy).

Bostryx sophieae Breure, 1979: 58 (replace-
ment name for *B. (B.) monticola* Doering)
[new synonymy].

Peronaeus (Peronaeus) cordillerae – Parodiz,
1946b: 315, pl. 1, fig. 4; 1947: 2; Fernández,
1973a: 88.

Peronaeus cordillerae – Parodiz, 1957b: 23;
1961: 60.

Bostryx cordillerae – Breure, 1978: 66, fig. 87;
1979: 52; Köhler, 2007: 132.

Type Locality: “Gola di Villa Vicencio e Casa
de Piedra nelle Preande Mendozine ...
Latitudine: 32° 33’”. This locality is located
in Mendoza Prov., Las Heras Dept.

Type Material: lectotype ZMB 24975a; paralec-
totypes ZMB 24975b (3).

Distribution: Argentina, *San Juan Prov.*,
Calingasta-Zonda Dept., Sierra del Tontal
(MACN-In 12114). *Mendoza Prov.* (RMNH
s/n, MACN-In 10001-2 A, MACN-In 1354), Las
Heras Dept., Quebrada del Toro (MLP 897,
MLP 9669, MLP 9660, MACN-In 36944 A),
Casa de Piedra (ZMB 24975b), Cerro Pelado

(MLP 39802, MACN-In 9592 A), Quebrada de las Pircas (MACN-In 32867 A), Uspallata (MACN-In 19625 A, MACN-In 9849, MACN-In 9850), Villavicencio (IFML 15580, MACN-In 3234, MLP 8062, ZMB 24975a), Cerro El Arco (MCNL 361).

Remarks

B. cordillerae is synonymized with *B. sophieae* based on its shell morphology. Shell measurements of the lectotype fits into *B. cordillerae* size range. Moreover, area of distribution of *B. sophieae* is included in the area of occurrence of *B. cordillerae*.

Bostryx costellatus
(Hylton Scott, 1971)
Fig. 1D

Bulimulus (Scansicochlea) costellatus Hylton Scott, 1971: 77.

Bostryx costellatus – Breure, 1979: 53.

Bostryx birabenorum – Miquel, 1993: 158, pl. 1, fig. 2 [*partim*].

Type Locality: “Cerro de Cachi, Prov. de Salta”.

Type Material: holotype MACN-In 4221-1; paratypes MACN-In 4221-5 (3).

Distribution: Argentina, *Salta Prov.*, Cachi Dept. (MACN-In 4221-1, MACN-In 4221-5).

Remarks

Holotype label is handwritten by Hylton Scott under the name “*Scansicochlea costulata* Hylton Scott”, lot number MACN-In 4221.1. However, in the original description, the species name was *Bulimulus (Scansicochlea) costellatus*. Hylton Scott indicated that J. J. Parodiz gave this provisional name [*Scansicochlea costulata*] to this lot before she described it as a new species with the name *Bulimulus (Scansicochlea) costellatus*.

Bostryx cuyanus
(Pfeiffer, 1867)

Helix cuyana Pfeiffer, 1867a: 79; – Pfeiffer, 1867–1869b: 332, pl. 79, figs. 16–18.

Helix (Lysinoe) cuyana – Strobel, 1874: 11, pl. 1, fig. 2.

Helix cuyana – Doering, 1874a: 53.

Epiphragmophora cuyana – Doering, 1874b [1875]: 448.

Bostryx (Platybostryx) cuyana – Hylton Scott, 1954: 409.

Bostryx (Platybostryx) doelloi Hylton Scott, 1954: 412.

Platybostryx cuyana – Parodiz, 1957b: 24; 1961: 61; Fernández 1973a: 99.

Platybostryx doelloi – Parodiz, 1957b: 24; 1961: 61; Fernández 1973a: 100.

Bostryx cuyanus – Breure, 1978: 71, pl. 10, figs. 7, 11; 1979: 53; Miquel, 1993: 159, pl. 1, figs. 3, 4, pl. 2, fig. 16.

Bostryx lentiformis Breure, 1978: 96; – Breure, 1979: 55.

Bostryx doelloi – Breure, 1979: 53.

Type Locality: “Argentina, Mendoza Prov.”.

Type Material: Not located.

Bostryx (Platybostryx) doelloi: paratypes MACN-In 9592-3 (12), MACN-In 10000 (4), MLP 10980 (3), MLP 10981 (1), MLP 41801 (2); *Bostryx lentiformis*: holotype, MACN-In 12113a, paratypes, MACN-In 12113 (9), RMNH 55325 (2).

Distribution: Argentina, *Mendoza Prov.* (MCZ 141259), Las Heras Dept. (MACN-In 10000, IFML 15581, IFML 11010), Cerro Pelado (MLP 41801, MLP 10980, MACN-In 9592-3, MACN-In 10000, MCNL 1117), Guamparito (MACN-In 30498 A), Las Cuevas (MLP 10981), Uspallata (MACN-In 8845). *San Juan Prov.*, Sierra del Tontal (MACN-In 12113a, MACN-In 12113, RMNH 55325).

Bostryx famatinus
(Doering, 1879)
Fig. 1E

Bulimulus (Peronaeus) famatinus Doering, 1879: 63.

Bulimulus (Bostryx-Peronaeus) famatinus – Pilsbry, 1896: 152.

Peronaeus (Peronaeus) famatinus – Parodiz, 1946b: 315, 322, fig. 13; 1947: 7; Fernández, 1973a: 88.

Peronaeus famatinus – Parodiz, 1957b: 22.

Bostryx famatinus – Breure, 1979: 53; Miquel, 1995: 121, pl. 1, fig. 3.

Type Locality: “Sierra de La Rioja, en cuesta situada entre esta ciudad y Chilecito, a una altura de cerca de 2000 m”. The type locality as it is described by Doering is probably the Sierra de Velasco, a mountain system located between Sanagasta and Chilecito Depts. in La Rioja Prov., Argentina.

Type Material: lectotype MACN-In 3233, paralectotypes MACN-In 3233-1 (3) [present designation]. Lectotype: H = 17.02, DM = 5.59, Hap = 7.19, Dap = 4.13.

Distribution: Argentina, *La Rioja Prov.*, Sana-gasta Dept. (MACN-In 3233).

Bostryx mendozanus
(Strobel, 1874)

Bulimulus (Eudiotus) mendozanus Strobel, 1874: 23, pl. 1 fig. 4; – Doering, 1879: 70.

Bulimus (Eudiotus) Mendozanus – Doering, 1875a [1877]: 340.

Bulimulus (Bulimulus) mendozanus – Pilsbry, 1897: 71, pl. 11, figs. 22, 23.

Peronaeus (Lissoacme) mendozanus – Parodiz, 1946b: 315; 1947: 26; 1957b: 23; 1961: 60; Fernández, 1973a: 94.

Bulimulus mendozanus – Breure, 1979: 63.

Bostryx mendozanus – Miquel, 1995: 121, pl. 1, fig. 4.

Type Locality: “Gola di Villa Vicencio, nelle Preande o Sierra de Mendoza, 1866... Latitudine: 32°–33°... Altezza: 1500–1800 m”. This type locality is located in Las Heras Dept. Mendoza Prov.

Type Material: syntype ZMB 24976 (1).

Distribution: Argentina, *Mendoza Prov.*, Las Heras Dept., Angostura de Villavicencio (ZMB 24976), Cordillera de Uspallata, Cerro de La Leña (MACN-In 9850, MACN-In 36977 A, MLP 8082).

Bostryx pastorei
(Holmberg, 1912)
Fig. 1F

Bulimulus (Mesembrinus) pastorei Holmberg, 1912a: 22.

Bulimulus (Scansicochlea) pastorei – Parodiz, 1946b: 315, 322, fig. 9, pl. 1, fig. 8; 1957a: 134; Fernández, 1973a: 85.

Peronaeus (Lissoacme) puntanus Parodiz, 1947: 13.

Bostryx puntanus – Breure, 1979: 57.

Bostryx pastorei – Miquel, 1995: 121, pl. 1 figs. 5, 6.

Type Locality: “Provincia de San Luis: en el Cerro Varela”. Cerro Varela is located in Capital Dept. of San Luis Prov.

Type Material: lectotype MACN-In 1517; paralectotypes MACN-In 1517-1 (6) [present designation]. Lectotype: H = 23.0, DM = 9.6, Hap = 10.68, Dap = 6.45; paralectotype 1: H = 19.92, DM = 9.14, Hap = 10.55, Dap = 6.03; paralectotype 2: H = 21.33, DM = 9.43, Hap = 10.77, Dap = 6.51; paralectotype 3: H = 19.2, DM = 8.74, Hap = 9.92, Dap = 6.05; paralectotype 4: H = 22.92, DM = 9.15, Hap = 10.79, Dap = 6.68.

Peronaeus (Lissoacme) puntanus: holotype, MACN-In 9917, paratypes, MACN-In 9917-1 (70), MACN-In 1316 (10), MACN-In 9834a (5).

Distribution: Argentina, *San Luis Prov.*, Coronel Pringles Dept., Carolina (MACN-In 28800), Cerro Sololasta (MACN-In 9834, MLP 9671), General Pedernera Dept., Cerro del Morro (MACN-In 9917, MACN-In 9917-1, MACN-In 1316), La Capital Dept., Cerro Varela (MACN-In 1517, IFML 15582), San Martín Dept., quebrada between Quines and Libertador San Martín (IFML 15573).

Bostryx reedi
(Parodiz, 1947)
Fig. 1G

Peronaeus (Lissoacme) reedi Parodiz, 1947: 10; – Parodiz, 1957b: 24; 1961: 61; Fernández, 1973a: 95.

Bostryx reedi – Breure, 1979: 57; Miquel, 1993: 162, pl. 1, fig. 6, pl. 2, fig. 15.

Type Locality: “La Cueva, [no Las Cuevas], provincia de Mendoza, Argentina”.

Type Material: holotype MACN-In 10001.

Distribution: Argentina, *Mendoza Prov.*, La Cueva (MACN-In 10001, MACN-In 10001-1 A), Las Heras Dept., Cerro Pelado (MACN-In 1116, MACN-In 9592-1 A), Guamparito (MACN-In 10001).

Bostryx rudisculptus
(Parodiz, 1956)
Fig. 1H

Bulimulus (Scansicochlea) rudisculptus Parodiz, 1956b: 78, fig. 7; – Fernández, 1973a: 85.

Bostryx rudisculptus – Breure, 1979: 58.

Bostryx tortoratus – Miquel, 1995: 123 [partim].

Type Locality: "Baños de Villavil, provincia de Catamarca". The location of the type locality is within Belén Dept. in Catamarca, Argentina.

Type Material: holotype MACN-In 380; paratypes MACN-In 380 (5).

Distribution: Argentina, *Catamarca Prov.*, Belén Dept., Baños de Villavil (MACN-In 380, MACN-In 380-1, MLP 10171).

Remarks

Bostryx rudisculptus is a species originally described in *Bulimulus* by Parodiz (1956b). Later, the Parodiz (1957) did not listed *Bulimulus* (*Scansicochlea*) *rudisculptus* as a species present in Argentina. Fernandez (1973) mentioned that *B. rudisculptus* is distributed in Catamarca province.

Bostryx stelzneri (Dohrn, 1875)

Bulimulus (*Scutalus*) *stelzneri* Dohrn, 1875: 202; 1877: 157; Doering, 1879: 66; Holmberg, 1912a: 23.

Bulimus (*Scutalus*) *Stelzneri* – Doering, 1875a [1877]: 339.

Bulimulus stelzneri – Pfeiffer, 1876: 58; Kobelt, 1878: 149, pl. 6, fig. 7.

Bulimulus (*Scutalus*) *conispirus* Doering, 1879: 67.

Bulimulus (*Scutalus*) *conispirus fasciata* Doering, 1879: 67.

Bulimulus (*Bostryx-Lissoacme*) *conispirus* – Pilsbry, 1896: 189 [incorrect subsequent spelling].

Bulimulus (*Bostryx-Lissoacme*) *stelzneri* – Pilsbry, 1896: 190, pl. 51, figs. 20–22.

Bulimulus (*Thaumastus*) *hector* Holmberg, 1909a: 11; 1912b: 149.

Bulimulus hector var. *cora* Holmberg, 1909a: 12.

Bulimulus hector var. *helenae* Holmberg, 1909a: 12.

Bulimulus hector var. *giovanni* Holmberg, 1909a: 12.

Bulimulus hector var. *montana* Holmberg, 1909a: 12.

Bulimulus hector var. *cactorum* Holmberg, 1909a: 12.

Bulimulus stelzneri hector – Hylton Scott, 1945: 204.

Neopetraeus stelzneri – Parodiz, 1946b: 315, fig. 5, pl. 3, figs. 1–12; 1948: 7; 1957a: 134; Zilch, 1971: 198; Fernández, 1973a: 106.

Neopetraeus stelzneri apertus Hylton Scott, 1948a: 238; Parodiz, 1957a: 135; Fernández, 1973a: 109.

Neopetraeus stelzneri f. *hybrida* Parodiz, 1948: 12, pl. 3, fig. 11; – Fernández, 1973a: 107.

Neopetraeus stelzneri f. *nonogastanus* Parodiz, 1948: 13, pl. 3, fig. 1; – Fernández, 1973a: 107.

Neopetraeus stelzneri f. *tinogastanus* Parodiz, 1948: 13; – Fernández, 1973a: 108.

Neopetraeus stelzneri peristomatus f. *paraconispirus* Parodiz, 1948: 16; – Fernández, 1973a: 112.

Neopetraeus stelzneri var. *conispirus* – Parodiz, 1948: 16.

Neopetraeus stelzneri var. *hector* – Parodiz, 1948: 18.

Neopetraeus stelzneri conispirus f. *minuta* Parodiz, 1948: 18; – Fernández, 1973a: 110.

Neopetraeus stelzneri hector – Parodiz, 1957a: 135; Fernández, 1973a: 110.

Neopetraeus stelzneri conispirus – Parodiz, 1957a: 134; Fernández, 1973a: 109.

Neopetraeus stelzneri hector f. *multicincta* – Parodiz, 1948: 20; Fernández, 1973a: 110.

Neopetraeus stelzneri hector f. *tricincta* – Fernández, 1973a: 111.

Bostryx stelzneri apertus – Breure, 1979: 51.

Bostryx conispirus – Breure, 1979: 52.

Bostryx hector – Breure, 1979: 54.

Bostryx stelzneri nonogastanus – Breure, 1979: 56.

Bostryx stelzneri tinogastanus – Breure, 1979: 59.

Bostryx stelzneri – Breure, 1978: 127, figs. 191–199; 1979: 58; Miquel, 1993: 163, pl. 1, figs. 9, 10, 12, pl. 2, figs. 13, 14 [partim].

Type Locality: "Cerro de Chepe". Cerro Chepe is located in Argentina, La Rioja Prov., Rosario Vera Peñaloza Dept.

Type Material: Not located.

Neopetraeus stelzneri apertus: holotype, MLP 11292, paratype, MACN-In 26645 (1);

Neopetraeus stelzneri conispirus f. *minuta*: holotype, MACN-In 6243, paratypes, MACN-In 6243-1 (26), MLP 1298 (1);

Neopetraeus stelzneri hybrid: holotype, MACN-In 433; paratypes, MACN-In 433-1 (45), MLP 1303 (2);

Neopetraeus stelzneri f. *nonogastanus*: holotype, MACN-In 17591, paratypes, MACN-In 17591-1 (10), MACN-In 17613 (10);

Neopetraeus stelzneri peristomatus f. *paraconispirus*: holotype, MACN-In 14086, paratypes, MACN-In 14086-1 (7);

Neopetraeus stelzneri f. *tinogastanus*: holotype, MACN-In 19061, paratypes, MACN-In 19061-1 (6).

Distribution: Bolivia; Argentina, *Catamarca Prov.*, Ambato Dept., Sierra de Ambato (MACN-In 9682, MLP 9538), Andalgalá Dept., Agua de Las Palomas (IFML 15591), Cuesta de Andalgalá (MLP 9529, MLP 9533), Quebrada de Arenal (MACN-In 17850); Belén Dept., Cuesta de Belén (IFML 15526, MACN-In 21116 A, MLP 9531, MLP 9536), Cuesta el Tambillo (IFML 11018), Hualfín (IFML 14925), Quebrada de Indalecio (IFML 15461 A), Villa Vil (MACN-In 382, MLP 9518); Capayán Dept., Chumbicha (MACN-In 9229 A), Concepción (MACN-In 9227, MACN-In 17777 A); Esquiú Dept., Las Pirquitas dam (IFML 15459 A); Santa María Dept., El Desmonte (IFML 14447); Tinogasta Dept. (IFML 11002, IFML 11020), Cuesta de La Chilca (IFML 15592, IFML 15594, IFML 1616, IFML 11019, IFML 78, IFML 14443, IFML 15283, IFML 15473 A, IFML 15474 A, IFML 15475 A, IFML 15593); Cuesta de Zapata (MLP 803, IFML 10896, IFML 14446, IFML 15527, IFML 15515, IFML 15460 A), La Coipita (MACN-In 19061, MACN-In 19061-1), Santa Rosa (MLP 9515), Sierra de Fiambalá (MACN-In 19619, MLP 1298). *Cordoba Prov.*, Cruz del Eje Dept., Aguas de Ramón (MACN-In 32849 A). *Jujuy Prov.* (IFML 506), Dr. Manuel Belgrano Dept., before of Quebrada de Humahuaca (IFML 15481 A), Humahuaca Dept., Coctaca (MACN-In 2466 A, MLP 9527), Maimará (MACN-In 19590), Quebrada de Humahuaca (MACN-In 586 A, MACN-In 35642, MACN-In 19506 A, IFML 15469 A, MLP 9523), Río Humahuaca (MACN-In 19460), Uquía (MACN-In 8838 A, MACN-In 12335 A); Santa Bárbara Dept., Puesto Viejo (MACN-In 988); Tilcara Dept. (IFML 15585, IFML 984, MLP 1299, MLP 9522, MLP 9519, MLP 9524, MACN-In 586 A, MACN-In 1629), Cerro Negro (IFML 14878, IFML 15468 A, IFML 15516, IFML 16467 A, MACN-In 1636 A), Cerro Pucará (MACN-In 30615, MACN-In 30618 A, MACN-In 19506 A, MACN-In 19525 A, MACN-In 13285 A), Garganta del Diablo (IFML 5584, IFML 560, IFML 15466 A, IFML 15518), Huacalera (IFML 10449, IFML 15482 A, IFML 15589), Sierra de Tilcara (IFML 410); Tumbaya Dept. (IFML 15583, IFML 15587, IFML 15470 A, MACN-In 30597 A), Quebrada de Purmamarca (MLP 9517), Volcán (MACN-In 24308 A). *La Rioja Prov.* (IFML 15590, MACN-In 19946), Arauco Dept., Aimogasta (MACN-In 7810 A, MACN-In 24398 A); Castro Barros Dept., Chuquis (MLP 9534), Coronel Felipe Varela Dept., Cuesta de Miranda (MACN-In 434, MACN-In 30441), Sierra de Velázquez (IFML 15456

A); Chilecito Dept. (IFML 11000, IFML 11001, IFML 15513, MLP 9539), Nonogasta (MACN-In 17613, MACN-In 17591 A), Sañogasta (IFML 467, IFML 12764); Famatina Dept. (MACN-In 17849 A, IFML 15514, IFML 15458 A); General Belgrano Dept., Iliar (MACN-In 18356 A), Olta (MACN-In 18325, MLP 1300); Independencia Dept., El Chiflón (MLP 9514), La Torre (IFML 11045, IFML 15586), Patquia (MLP 9535); Sanagasta Dept. (MLP 9521), Quebrada de Los Sauces (MACN-In 15703 A, IFML 15457 A), Quebrada de Sanagasta (MACN-In 9852); San Blas de los Sauces Dept., Cerro de Velazco (MLP 1303, MACN-In 433, MACN-In 433-1). *Salta Prov.* (MACN 17597 A), Chicoana Dept., Quebrada de Agua Negra (MLP 2393); Guachipas Dept. (MACN-In 17595), Alemanía (MACN-In 582 A, MACN-In 17596, MACN-In 17597, MLP 1535, MLP 1545); La Viña Dept., Quebrada de Guachipas (MACN-In 3218); Metán Dept., Cerro Colorado (MACN-In 32861, MACN-In 36890, MACN-In 26522, MACN-In 26229, MLP 9528), Dique Cabra Corral (IFML 15544 A, IFML 15545 A, IFML 15543 A), Pala Pala (IFML 15492 A); Rosario de la Frontera Dept. (MLP 1307); Rosario de Lerma Dept. (IFML 15463 A), between Alfarcio and Santa Rosa de Tastil (IFML 15464 A), El Golgota (IFML 15588, IFML 14072), Ruinas de Santa Rosa de Tastil (IFML 15455 A). *San Juan Prov.*, Jáchal Dept., Cerro Agua Negra (MACN-In 24305), Ciénaga (MACN-In 10655); Zonda Dept., Cerro Negro (MACN-In 9920). *San Luis Prov.* (MACN-In 15711), Ayacucho Dept., Luján (MACN-In 9834), Santo Domingo (MLP 9516-1), Sierra de Quines (MACN-In 15122); Belgrano Dept., Sierra Gigante (MACN-In 14086, IFML 14086-1); Coronel Pringles Dept., La Toma (MACN-In 1018 A), Las Cañas (MACN-In 2426); Libertador General San Martín Dept., Quebrada entre Quines y Libertador San Martín (IFML 15462 A). *Tucuman Prov.* (MACN-In 9159, IFML 10505), Tafí del Valle Dept. (IFML 71), Amaicha del Valle (MLP 1542), Ruinas de Quilmes (IFML 15465 A), Tafí Viejo Dept., Las Arquitas (MACN-In 10505).

Remarks

Bostryx stelzneri is characterized by its shell polymorphism and a very wide geographical distribution in Argentina. Due to its high shell variability, several *varieties* and *forms* have been described by different authors for this species, *Bostryx stelzneri stelzneri* (Dohrn, 1875),

Bostryx stelzneri peristomatus (Doering, 1879), *Bostryx stelzneri conispirus* (Doering, 1879), *Bostryx stelzneri hector* (Holmberg, 1909), *Bostryx stelzneri apertus* (Hylton Scott, 1948), *Bostryx stelzneri hybrida* (Parodiz, 1948), *Bostryx stelzneri nonogastanus* (Parodiz, 1948), *Bostryx stelzneri tinogastanus* (Parodiz, 1948) and *Bostryx stelzneri scaber* (Parodiz, 1948) (Fig. 1I). *Bulimulus hector* Holmberg, 1909, *Bulimulus (Scutalus) peristomatus* Doering, 1879 and *Bulimulus (Scutalus) conispirus* Doering, 1879 were originally described with the status of species, but later considered as varieties of *B. stelzneri* by Parodiz (1948). Here, we cited *Bostryx stelzneri sensu lato* including all subspecies described awaiting a taxonomic revision of this species complex.

Bostryx strobeli
(Parodiz, 1956)
Fig. 1J

Bulimulus (Scansicochlea) strobeli Parodiz, 1956a: 62; – Fernández, 1973a: 85.

Bulimulus (Scansicochlea) lolae Hylton Scott, 1967b: 11.

Bostryx lolae – Breure, 1979: 55.

Bostryx strobeli – Breure, 1979: 58; Miquel, 1995: 122, pl. 1, figs. 7, 8.

Type Locality: “Cerro del Morro, provincia de San Luis”. The type locality is located in the General Pedernera Dept, San Luis province.

Type Material: holotype MACN-In 9916; paratypes MACN-In 9916-1 (4), MLP 11018 (2). *Bulimulus (Scansicochlea) lolae*: paratypes MACN-In 27283 (1), MLP 11010 (1), MLP 11009 (1).

Distribution: Argentina, *Cordoba Prov.*, Calamuchita Dept., Santa Rosa de Calamuchita (MACN-In 27283, MLP 11009, MLP 11010). *San Luis Prov.*, General Pedernera Dept., Cerro del Morro (MACN-In 9916, MACN-In 9916-1, MLP 11018, MLP 11449); Libertador General San Martín Dept., Cerro Inti Huasi (MLP 895).

Bostryx torallyi
(d’Orbigny, 1835)

Helix torallii d’Orbigny, 1835: 11.

Bulimulus montagnei d’Orbigny, 1837 [1834–1847]: pl. 32, figs. 5–7; 1838: 286; – Parodiz, 1946b: 322, fig. 8.

Bulimus montagnei – Gray, 1854: 17; Reeve, 1849: pl. 23, fig. 146.

Bulimus torallyi – d’Orbigny, 1837 [1834–1847]: 285, pl. 32, figs. 1–4; Gray, 1854: 17.

Bulimus draparnaudi Pfeiffer, 1846b: 113.

Bulimus (Mesembrinus) Torallyi – Parravicini, 1894: 5.

Bulimulus toralyis – Ancey, 1897: 5 [incorrect subsequent spelling].

Drymaeus torallyi – Pilsbry, 1898: 278, pl. 44, figs. 97–99, 1–3; Zischka, 1953: 82.

Bulimulus (Drymaeus) nigroumbilicatus Preston, 1907: 491, fig. 6.

Bulimulus thorallyi – Holmberg, 1909c: 91; Breure, 1975: 1146, pl. 8, fig. 4 [incorrect subsequent spelling].

Bulimulus (Mesembrinus) climacographus Holmberg, 1912b: 147, figs. 1, 2.

Peronaeus (Lissoacme) climacographus – Parodiz, 1946b: 315, pl. 2, fig. 7; 1947: 15.

Peronaeus (Lissoacme) torallyi – Parodiz, 1946b: 315, 322, fig. 10, pl. 1, fig. 1; 1947: 16; Fernández, 1973a: 95.

Peronaeus (Lissoacme) birabeni Hylton Scott, 1948b: 272, figs. 4–10.

Peronaeus (Lissoacme) torallyi avus Parodiz, 1947: 20; – Parodiz, 1957b: 23; Fernández, 1973a: 96.

Peronaeus (Lissoacme) torallyi corrugatus Parodiz, 1947: 19; – Parodiz, 1957b: 23; Fernández, 1973a: 96.

Peronaeus (Lissoacme) torallyi draparnaudi Parodiz, 1947: 18; – Parodiz, 1957b: 23; Fernández, 1973a: 97; Quintana, 1982: 92.

Peronaeus (Lissoacme) torallyi nigroumbilicatus Parodiz, 1947: 20; – Parodiz, 1957b: 23; 1962: 449; Fernández, 1973a: 97; Quintana, 1982: 93.

Drymaeus draparnaudi – Breure, 1975: 1159.

Bostryx climacographus – Breure, 1978: 60, fig. 79; 1979: 52.

Bostryx torallyi – Breure, 1979: 59; Miquel, 1995: 122, pl. 1, figs. 9–11.

Peronaeus (Lissoacme) torallyi torallyi – Quintana, 1982: 92.

Bostryx birabeni – Neubert & Janssen, 2004: 202, pl. 6, fig. 69 [non Hylton Scott, 1948].

Type Locality: “provincia Valle-Grande (republica Boliviana)”. In 1837 [1834–1847] d’Orbigny increased the information concerning the type locality of this species detailing that: “Nos avons rencontré le Bulime de Torally sur les cotaux escarpés et sees du Rio Grande dans les provinces de la Laguna et de Valle Grande ... Cordillères boliviennes” “Cette espèce abonde, surtout, au lieu nommé Pampa Ruiz”.

Type Material: lectotype MNHN 23251; paralectotypes MNHN 23252 (3), MNHN 23253 (3), MNHN 23254 (1), MNHN 23255 (5), SMF 171805 (1).

Peronaeus (Lissoacme) birabeni: holotype, MLP 11329, paratypes, MACN-In 32639 (1), MACN-In 6969 (2); *Peronaeus (Lissoacme) torallyi avus*: holotype, MACN-In 3236, paratypes, MACN-In 3236-1 (3); *Peronaeus (Lissoacme) torallyi corrugatus*: holotype, MACN-In 25987, paratypes, MACN-In 25987-1 (10); *Peronaeus (Lissoacme) torallyi nigroumbilicatus*: holotype, MACN-In 8848, paratypes MACN-In 8848-1 (14); *Bulimulus (Mesembrinus) climacographus*: holotype, MACN-In 1349, paratype, MACN-In 1349-1 (1); *Bulimulus montagnei*: syntypes NHMUK 1854.12.4.194 (8).

Distribution: Bolivia; Paraguay; Perú; Argentina, Chaco Prov., Almirante Brown Dept., Taco Pozo (IFML 10526). Jujuy Prov. (IFML 588, MLP 9575), Ledesma Dept. (IFML 15500 A, IFML 15525), Urundel (IFML 903), Santa Bárbara Dept., Puesto Viejo (MACN-In 986). Salta Prov. (MLP 9676, MLP 9968, IFML 15598, IFML 15599); Anta Dept., Cañada de los Colorados (MACN-In 367), Quebrachal (MACN-In 19167, MLP 9680); Cachi Dept. (MACN-In 3236, MACN-In 3236-1); General Güemes Dept. (MACN-In 36937 A), Cerro Pelado (IFML 387), Las Mesitas (IFML 15603); General José de San Martín Dept., Embarcación (MLP 11329, MLP 11330, MLP 11331, MLP 11332, IFML 15604, MACN-In 8848, MACN-In 8848-1, MACN-In 32639, MACN-In 6969, MACN-In 32645, IFML 120, IFML 12759, IFML 12757, IFML 855), near Tartagal (IFML 15602); La Candelaria Dept., Quebrada del Río Ceibal (IFML 15596), Santa Bárbara (IFML 122); Metán Dept., between Lumbrera and Juramento (IFML 579), Cerro Colorado (MACN-In 26230, MACN-In 26231, MACN-In 26518, IFML 15597, MLP 9667, MLP 9966, MLP 9681), Lumbrera (IFML 876, IFML 15498 A, IFML 15600), route to Dique Cabra Corral (IFML 15497 A, IFML 15499 A, IFML 15547 A, IFML 15548, IFML 15549, IFML 15575, IFML 15576, IFML 15577, IFML 15578, IFML 15579). Santiago del Estero Prov., Pellegrini Dept., Cerro del Remate (IFML 132). Tucuman Prov. (IFML 11305), Burruyacu Dept., Río Nío (IFML 10933); Juan Bautista Alberdi Dept., Escaba (IFML 15523, IFML 15524, IFML 15496 A); La Cocha Dept., Dique San Ignacio (IFML 11302); Lules Dept., Villa Nougues (MACN-In 18363, MACN-In 25987, MACN-In 25987-1,

MLP 9679); Tafí Viejo Dept., El Cadillal (MLP 9659); Trancas Dept., Arroyo India Muerta (IFML 15605, IFML 974, IFML 15494 A, IFML 15493 A, IFML 15495 A, IFML 15528), Tapia (IFML 15522, MACN-In 1349, MACN-In 1349-1, MACN-In 9837, MACN-In 23049), Vipos (IFML 160, IFML 191, MACN-In 18319, MACN-In 17594, MACN-In 17578, MACN-In 21475, IFML 15606, MLP 9678).

Remarks

Bostryx torallyi was originally described by d'Orbigny (1835) in the genus *Helix* Linnaeus, 1758. Subsequently classified in *Bulimus* by d'Orbigny (1837, 1838 [1834–1847]), in *Bulimulus* Leach, 1814, by Parravicini (1894) and in *Drymaeus* Albers, 1850, by Pilsbry (1897–1898) on the grounds of shell characters. Breure (1979) and Miquel (1995) relocated *B. torallyi* in *Bostryx*. This species shows high shell variability in form and coloration and mainly for this reason three subspecies have been described by Parodiz (1947), *Peronaeus (Lissoacme) torallyi corrugatus*, *Peronaeus (Lissoacme) torallyi avus* and *Peronaeus (Lissoacme) torallyi nigroumbilicatus*.

Miquel (1995) synonymized all Parodiz subspecies.

Bostryx tortoranus (Doering, 1879)

Bulimulus (Bulimulus) tortoranus Doering, 1879: 71.

Bulimulus (Bostryx-Lissoacme) tortoranus – Pilsbry, 1896: 192.

Bulimulus jujuyensis Holmberg, 1909a: 11; – Holmberg, 1912b: 149; Hylton Scott, 1945: 207, fig. 236; Parodiz, 1946b: 315; 1957a: 134; 1962: 434; Breure, 1978: 144.

Peronaeus (Lissoacme) tortoranus – Parodiz, 1946b: 315; 1947: 12; 1957b: 23; Fernández, 1973a: 98.

Bulimulus (Scansicochlea) hyltonscottae Parodiz, 1956a: 59, figs. 1–5.

Bulimulus (Scansicochlea) martinezi Hylton Scott, 1965: 25.

Bulimulus (Scansicochlea) cicheroi Hylton Scott, 1967b: 7.

Bulimulus (Scansicochlea) gladysae Hylton Scott, 1967b: 8.

Bostryx martinezi – Breure, 1978: 100, figs. 148–149.

Bostryx cicheroi – Breure, 1979: 52.

Bostryx gladysae – Breure, 1979: 54.

Bostryx hyltonscottae – Breure, 1979: 54.

Bostryx tortoranus – Breure, 1979: 59; Miquel, 1995: 123, pl. 1, figs. 12–17.

Type Locality: "...quebradas húmedas de la sierra de Pocho (Totoras, Yatan, Cerro Salado)". The mountain system "sierra de Pocho" is located in Cordoba Prov., Pocho Dept.

Type Material: Not located.

Bulimulus (Scansicochlea) gladysae: holotype, MLP 13345, paratypes, MLP 11007 (1), MLP 11442 (1), MLP 11006 (1), MLP 11444 (1), MLP 11443 (1), MACN-In 27282 (4); *Bulimulus (Scansicochlea) hyltonscottae*: holotype, MACN-In 6516, paratypes, MACN-In 6516-1 (4); *Bulimulus (Scansicochlea) cicheroi*: holotype, MLP 10996, paratypes, MLP 10995 (1), MLP 10997 (1), MACN-In 27281 (1); *Bulimulus (Scansicochlea) martinezi*: holotype, MLP 11448, paratypes, MACN-In 27208 (3), MLP 11011 (1), MLP 11012 (1), MLP 11446 (1), MLP 11447 (1); *Bulimulus jujuyensis*: holotype, MACN-In 32686; *Bulimulus (Bulimulus) monticola*: syntypes ZMB 34734 (2).

Distribution: Argentina, *Catamarca Prov.* (MLP 11442, MLP 11443; MLP 11444, MLP 13345, MLP 11006, MLP 11006), Andalgalá Dept., Cuesta de la Chilca (MLP 11442, MLP 11444, MLP 11443, MLP 11445, MLP 11006, MLP 11007, MLP 13345, MACN-In 27282). *Cordoba Prov.* (MLP 9670), Pocho Dept., Chancani (MLP 8080, MLP 8081, MLP 11447, MLP 11012), between Las Palmas and Chancani (IFML 15608, IFML 15611, IFML 15610, IFML 15609, MACN-In 27208, MACN-In 27431, MACN-In 36892 A, MLP 10169, MLP 10170, MLP 11448, MLP 11011, MLP 11012, MLP 11446, MLP 11447), between Las Palmas and El Cadillo (IFML 15506 A, IFML 15507 A, IFML 15508 A). *Jujuy Prov.* (MLP 1534, MLP 8076, MLP 8077), Ledesma Dept., Fraile Pintado (MLP 8071); Tilcara Dept. (MACN-In 19506, MACN-In 19507, MACN-In 32686, MLP 8070, MLP 8075, MLP 1256-1, MLP 1526-1); Cerro Negro (IFML 14877 A, IFML 15530, IFML 15001 A, IFML 15574 A), route from Tilcara to Garganta del Diablo (IFML 158, IFML 15607); Tumbaya Dept. (MLP 8078). *Salta Prov.*, Cachi Dept. (IFML 15505 A, IFML 15612); Cafayate Dept. (IFML 15248). *San Luis Prov.*, Ayacucho Dept., Luján (MACN-In 3235), El Zapallar (MACN-In 6516, MACN-In 6516-1), Junín Dept., Quebrada de Cautana (MLP 10996, MLP 10997, MACN-In 27281). *Tucuman Prov.*, Tafí del Valle Dept. (IFML 15282, IFML 15510 A), between Amaicha

and El Infiernillo (IFML 15281 A, IFML 15502 A, IFML 15503 A), Ruinas de Quilmes (IFML 15504 A).

Remarks

In 1995, Miquel sustained that *B. monticola* (= *B. sophiae*), *B. jujuyensis*, *B. sporadicus gracilis*, *B. hyltonscottae*, *B. rudisculptus*, *B. martinezi*, *B. cicheroi* and *B. gladysae*, are variations of *B. tortoranus* and synonymized them all based on the study of shell characters.

Type material and specimens from different localities of occurrences of this species were examined in the present study. *Bulimulus jujuyensis*, *B. hyltonscottae*, *B. gladysae*, *B. martinezi* and *B. cicheroi* have same shell characters and measurement than *B. tortoranus* thus are synonymized under *B. tortoranus*. However, *B. sophiae*, *B. sporadicus gracilis* and *B. rudisculptus* are different species from *B. tortoranus* and can be clearly differentiated by their shell shape and size.

Bostryx willinki

Weyrauch, 1964

Bostryx (Bostryx) willinki Weyrauch, 1964: 54, fig. 12; – Fernández, 1973a: 98.

Bostryx willinki – Breure, 1979: 60; Miquel, 1993: 164, pl. 1, figs. 7, 8.

Type Locality: "Argentina, prov. Catamarca, quebrada de Tinogasta".

Type Material: holotype IFML 121a; paratypes IFML 121 (3).

Distribution: Argentina, *Catamarca Prov.*, Tinogasta Dept. (IFML 121, MACN-In 19062).

Genus *Bulimulus*

Leach, 1814

Type Species: *Helix exilis* Gmelin, 1791 [original designation].

Geographic Distribution: Mexico, West Indies, Venezuela, Guyana, Surinam, French Guiana, Colombia, Panama, Costa Rica, Nicaragua, Guatemala, Belize, Brazil, Paraguay, Uruguay, Argentina, Bolivia, Peru, Ecuador.

Remarks

Fernández (1973) cited *Bulimulus gorritiensis* as a species distributed in Argentina, although

she did not state any specific locality of occurrence. After revision of all material deposited in the main Argentinean malacological collections, we did not find any historical or recently collected material of this species. Parodiz (1957a) and Miquel (1991) mentioned that *Bulimulus gorritiensis* only inhabits Uruguay. For this reason, we have not listed *B. gorritiensis* in this catalogue.

Bulimulus apodemetes
(d'Orbigny, 1835)

Helix (Cochlogena) apodemetes d'Orbigny, 1835: 10.

Bulimulus apodemetes – d'Orbigny, 1837 [1834–1847]: pl. 30, figs. 5–8; 1838: 279; Döering, 1879: 68; Parravicini, 1894: 6; Ancey, 1897: 5; Breure, 1978: 141, fig. 231; Miquel, 1991: 93, figs. 12–19, 45–46.

Bulimulus pessulatus Reeve, 1849: pl. 23, fig. 153.

Bulimulus (Bostryx-Lissoacme) apodemetes – Pilsbry, 1898: 187, pl. 54, figs. 1–4.

Bulimulus (Scutalus) riojanus Holmberg, 1912a: 23.

Bulimulus (Bulimulus) apodemetes – Parodiz, 1946b: 315, 318, fig. 2, pl. 2, fig. 1; Fernández, 1973a: 74; Breure, 1975: 1145; Quintana, 1982: 87.

Bulimulus apodemetes dispar Hylton Scott, 1952: 23, pl. 1, fig. 5; – Parodiz, 1957a: 134.

Bulimulus moei Parodiz, 1962: 431.

Type Locality: “republica Argentina; republica Boliviana”. In 1837 (1834–1847) d'Orbigny increased the information concerning to the type locality of this species detailing that *Bulimulus apodemetes* was found over “sur les coteaux du Parana, province d'Entre-rios, près de Feliciano au 31° degré de latitude sud ...”, “... près du convent de San-Lorenzo, province de Santa-Fe ...”, “... provinces de Valle grande et de la Laguna ...”.

Type Material: syntypes MNHN 24664 (4), Bolivia; MNHN 24663 (6), Bolivia, Pampa Grande; MNHN 24662 (4), Bolivia, Vilma.

Bulimulus pessulatus: syntypes NHMUK 1975313 (3), Bolivia; *Bulimulus apodemetes dispar*: paratype IFML 736 (1).

Distribution: Bolivia; Paraguay; Uruguay; Argentina, *Catamarca Prov.* (MLP 909, IFML 11054), *Ambato Dept.* (MACN-In 482); *Andalgalá Dept.* (IFML 202); *Belén Dept.* (IFML 774); *Fray Mamerto Esquiú Dept.*, *Las Pirqui-*

tas dam (MLP 8037); *El Alto Dept.* (IFML 567); *Paclín Dept.* (IFML 843). *Chaco Prov.*, *Añatuya* (MACN-In 12124); *Chacabuco Dept.*, *Charata* (MACN-In 17155), *Gancedo* (MACN-In 14524, MACN-In 13967). *Cordoba Prov.* (MLP 8014, MLP 32012, MLP 912, MLP 32005, MLP 8006, MLP 32004, MLP 8034, MLP 32017, MLP 8015, IFML 15648, IFML 15646, IFML 15644), *Calamuchita Dept.* (MLP 8028, MACN-In 3222, IFML 15650), *Río Tercero* (MLP 8010, MLP 8011, MACN-In 1871); *Colón Dept.*, (IFML 15647), *Cabana* (MLP 8038, MLP 907, MLP 10185-6), *Jesús María* (MLP 8036, 908, IFML 15649), *Unquillo* (MACN-In 30166); *Cruz del Eje Dept.*, *San Gregorio* (MLP 8007); *Ischilín Dept.* (IFML 10987), *La Calera* (MACN-In 475); *Punilla Dept.*, *Capilla del Monte* (MACN-In 9228), *Concepción* (MACN-In 17776), *Gutenberg* (MLP 8002), *Cosquín* (MACN-In 1000, MACN-In 9863), *Lago San Roque* (MACN-In 10426, MACN-In 14523); *San Alberto Dept.*, *Villa Brochero* (MACN-In 13254), *Villa María* (MACN-In 30611); *Santa María Dept.*, *Alta Gracia* (MLP 6686-1, MACN-In 20682); *Totoral Dept.*, (MLP 910), *Los Mistoles* (MLP 32002), *Macha* (MLP 8000), *Sierra Chica* (IFML 9), *Río Seco* (MACN-In 31624), *Saldán* (MLP 6696-4); *San Javier Dept.* (MLP 1317, MLP 2362, MLP 32015, MACN-In 3213, MACN-In 30638, MACN-In 11750), *Pucará* (MACN-In 11760); *Tulumba Dept.*, *San José de La Dormida* (MLP 8023, MLP 8012). *Entre Ríos Prov.*, *Paraná Dept.*, (MLP 2330, MLP 32013), *Paranacito* (MACN-In 20080). *Formosa Prov.*, *Matacos Dept.*, *Ingeniero Juárez* (MLP 8040, MLP 8020, MLP 8019, MACN-In 26523, MACN-In 18611), *Santa Victoria Dept.* (MLP 8025). *Jujuy Prov.* (MLP 2349, MLP 8009, IFML 14926, IFML 904, IFML 594, IFML 854, MACN-In 1343), *Las Capillas* (MACN-In 36940); *Ledesma Dept.*, *Calilegua* (MLP 8027); *San Pedro Dept.* (MLP 923, IFML 921, IFML 2901, IFML 11303), *El Palmar* (MLP 10685-1), *El Quemado* (MACN-In 9099), *Higueritas* (MLP 8003), *La Mendieta* (MLP 8033), *Pampa Blanca* (MACN-In 8113), *Perico del Carmen* (MACN-In 8112), *Puesto Viejo* (MACN-In 578, MACN-In 987). *La Rioja Prov.*, *Sanagasta Dept.*, *Quebrada de los Sauces* (MACN-In 15704), *Quebrada de Sanagasta* (MACN-In 1350); *General Belgrano Dept.* (MACN-In 18359). *Salta Prov.* (MLP 8022, MLP 8001, MLP 8013, MLP 8017, MLP 8016, IFML 848, IFML 584, IFML 702, IFML 12754, IFML 15654, IFML 15636, IFML 15634), *Guachipas Dept.*, *Alemanía* (MLP 32001, MACN-In 581, MACN-In 17596), *Gen-*

eral Güemes Dept. (MLP 32021, IFML 887), Campo Santo (IFML 658); Capital Dept., City of Salta (MACN-In 3064, MACN-In 30641, MACN-In 8105, IFML 15641), San Bernardo (MLP 32009, MACN-In 14774); San Martín Dept., Embarcación (MLP 920, MACN-In 8848, MACN-In 9340), La Candelaria (IFML 529, IFML 15640), Pocitos (MLP 10994, MLP 10993, MLP 10992, MLP 10991, MLP 10990, MLP 10989), Tartagal (MLP 8005); Orán Dept. (MLP 8029, IFML 15639), Manuel Elordi (MLP 8018), Tabacal (MACN-In 36880), Urundel (MLP 8021), Vespucio (MLP 8031, MLP 32014); Metán Dept. (MLP 8030, MACN-In 8104, IFML 10726, IFML 453, IFML 174, IFML 578, IFML 814, IFML 15642, IFML 15643), Juramento river (IFML 11304); Rosario de la Frontera Dept. (MACN-In 6189, MACN-In 10016, IFML 15626, IFML 15621); Rosario de Lerma Dept. (IFML 15637); Santa Victoria Dept. (IFML 15638), Pampa Grande (MACN-In 1325). Santa Luis Prov., San Martín Dept., Las Cañas (MACN-In 2445), Piedra Blanca (MLP 32020, 1279), Quebrada de Cautana (MLP 8026), Quines (MLP 868), Rincón (MACN-In 18314). *Santiago del Estero Prov.* (IFML 813, MACN-In 19692), Lazaro (MLP 2312); Banda Dept., Negra Muerta (MLP 913); Juan Ibarra Dept., Melero (MLP 32007); Ojo de Agua Dept., Dulce river (MACN-In 307); Pelligrini Dept. (IFML 15645); Guasayán Dept., Sierra de Guasayán (MACN-In 12951, IFML 767); Moreno Dept., Tintina (MLP 8032); Sarmiento Dept., Villa Matará (MACN-In 18248), Santa Catalina (MACN-In 29951). *Tucuman Prov.* (MACN-In 21477, IFML 15622, IFML 15628, IFML 15625, IFML 927, 459), Burruyacu Dept., (IFML 93, 457, 10992, 10699, 12393, 11301), Sierra del Nogalito (IFML 824); Capital Dept. (IFML 15651, IFML 1879, MACN-In 10182, MACN-In 10442, MACN-In 9699); Cruz Alta Dept., Las Cejas (IFML 13524); La Cocha Dept. (IFML 898, 963), San Ignacio river (IFML 969); Leales Dept. (IFML 15625, IFML 11223); Lules Dept., Villa Nogués (MACN-In 309); Juan Bautista Alberdi Dept. (IFML 15623); Tafí Viejo Dept. (IFML 14273), El Cadillal (MACN-In 14040, MACN-In 9007, MACN-In 9914, IFML 241), San Javier (MACN-In 9860); Trancas Dept., (IFML 11224, 328, 14921, 14922, 14923), Tapia (MACN-In 1335, MACN-In 8086), Timbó (MLP 32018), On the way to Jardin (IFML 11306), On the way to Raco (IFML 803, IFML 15624), On the way to Tapia (IFML 11297, IFML 15627), on the way to Tapia- Vipos (IFML 475, IFML 15635).

Bulimulus bonariensis
(Rafinesque, 1833)

- Syphalomphix bonariensis* Rafinesque, 1833: 165.
Helix sporadica d'Orbigny, 1835: 12.
Bulimulus sporadicus – d'Orbigny, 1837 [1834–1847]: pl. 32, figs. 12–15; 1838: 271; Parravicini, 1894: 6; Ancey, 1897: 5; Formica Corsi, 1900: 48; Hylton Scott, 1948a: 236.
Bulimulus gelidus Reeve, 1849: pl. 74, fig. 553.
Bulimulus montevidensis Pfeiffer, 1846a: 33.
Bulimulus (Thaumastus) sporadicus var. *bonariensis* – Strobel, 1874: 24; Pilsbry, 1897: 68, pl. 11, figs. 11–18.
Bulimulus sporadicus var. *montevidensis* – Pilsbry, 1897: 68.
Bulimulus heloicus fusca Ancey, 1901: 82.
Bulimulus (Drymaeus) morenoi Preston, 1907: 494, fig. 7.
Bulimulus? bonaerensis Holmberg, 1909: 10.
Bulimulus saltensis Holmberg, 1909c: 92.
Bulimulus (Bulimulus) sporadicus schadei Schlesch, 1935: 86, pl. 6, figs. 4, 5.
Bulimulus jujuyensis – Hylton Scott, 1945: 207 [non Holmberg 1909]; Breure, 1978: 144, fig. 236.
Bulimulus (Bulimulus) sporadicus – Parodiz, 1946b: 315.
Bulimulus (Bulimulus) bonariensis – Fernández, 1973a: 75.
Bulimulus bonariensis bonariensis – Parodiz, 1962: 432; Scarabino, 2003: 208.
Bulimulus (Bulimulus) bonariensis bonariensis – Fernández & Castellanos, 1973b: 278; Miquel, 1991: 98, fig. 5.
Bulimulus (Bulimulus) bonariensis sporadicus – Fernández, 1973a: 76; Breure, 1975: 1145; 1978: 142.
Bulimulus (Thaumastus) sporadicus bonariensis – Breure, 1979: 62.
Bulimulus bonariensis sporadicus – Quintana, 1982: 87; Miquel, 1991: 101, figs. 39–42.

Type Locality: “Buenos Ayres in South America”. All species described by Rafinesque came from a collection from Buenos Aires with no other detail about localities.

Type Material: Not located.

Distribution: Uruguay; Argentina, *Buenos Aires Prov.* (MACN-In 11711, MACN-In 17851, MACN-In 9826, MACN-In 29959), Adrogué (MACN-In 7059), Berisso (MLP 1530, MLP

2945, MLP 8057), between Otamendi and Campana (MACN-In 11890), Escobar (MACN-In 14611, MACN-In 18352), Campana (MACN-In 288, MACN-In 9922), Campo de Mayo (MACN-In 37177), Ensenada (MLP 12724), Ezeiza airport (MACN-In 23544), Gonzales Catán (MACN-In 30630), Islas del Tigre (MACN-In 12334, 7112), Junín (MACN-In 36260), La Copeta (MACN-In 7058, MLP 8048, MLP 8049), La Matanza river (MACN-In 29955), La Plata (MLP 8060), Luján (MACN-In 14608, MACN-In 287, MLP 2315), Lujan river (MACN-In 9856, MACN-In 37523), Magdalena (MLP 1913), Martin Garcia Island (MACN-In 597, MACN-In 566, MACN-In 7112), Obligado (MACN-In 37522), Olivos (MACN-In 16436, MLP 12486, MLP 8042), Palermo (MACN-In 281), Pergamino (MACN-In 285, MLP 8045), Puan (MACN-In 289), Punta Lara (MACN-In 11832, 11886), San Isidro (MLP 8046), San Nicolás (MLP 8059), Sierras de Cura Malal (MACN-In 1328), Villa Lugano (MACN-In 282), Zárate (MACN-In 16726), Zelaya (MACN-In 14207, MACN-In 14746). Chaco Prov. Basail (MACN-In 36979). *Cor-doba Prov.*, Monte Maiz (MLP 8041), Capital Dept. (MLP 8051, 8054, 8053). Corrientes Prov., Capital Dept. (MLP 8055, MACN-In 1345). Entre Ríos Prov., Paraná river (MACN-In 290), Gualeguaychú Dept., Gualeguaychú (MACN-In 36980, MLP 8043), Paraná Dept. (MACN-In 37774). Formosa Prov., Laishi Dept., Reserva Ecológica El Bagual (MACN-In 37785). Misiones Prov. La Candelaria Dept. (MACN-In 35897), Wanda (MACN-In 37737). Santa Fé Prov., Castellanos Dept., Clucellas (MACN-In 37011); General Obligado Dept. (MLP 8056); San Cristobal Dept. (MACN-In 37010), La Rubia (MLP 8044); San Javier Dept. (MLP 8050); Vera Dept., Caraguatay (MLP 8047). *Tucuman Prov.*, Capital Dept., San Miguel de Tucuman (MACN-In 10442).

Remarks

Rafinesque (1833) briefly described *Syphalomphix bonariensis* with the type locality “Buenos Ayres” in South America; *Helix sporadica* was described by d’Orbigny (1835) with type locality “provincia Corrientes (republica Argentina) and provincia Chiquitensis (republica Boliviana)”. These species are synonyms, as previously stated by Miquel (1991). In 1837 *Helix sporadica* was reallocated to *Bulimulus* by d’Orbigny and subsequently considered as a species related to *Bulimulus bonariensis*.

Confusedly, *sporadicus* and *bonariensis* names have been used alternatively in the taxonomic identification of specimens mainly collected in Buenos Aires and northeastern Argentina.

Strobel (1874) stated that *B. (T.) sporadicus* var. *bonariensis* is a variety that has a uniform coloration and that this species has affinities with *B. tenuissimus* (Férussac) and *B. (Mesembrinus) oreades* d’Orbigny. Parravicini (1894) stated that *B. sporadicus* is a species variable in shape but not in coloration and in shell whorl numbers. According to Pilsbry (1897–1898), typical *sporadicus* of d’Orbigny was restricted by Peiffer stating that has a shell brownish or whitish with brown longitudinal streaks. *Bulimulus bonariensis sporadicus* (d’Orbigny) is difficult to distinguish from *B. b. bonariensis* because they both share shell characters, similar external morphology, great coloration variability and have overlapping areas of distribution.

Bulimulus elatior
Hylton Scott, 1952
Fig. 1L

Bulimulus elatior Hylton Scott, 1952: 21, pl. 2, fig. 4; – Breure, 1979: 62.

Bulimulus eliator – Parodiz, 1957a: 134; Fernández, 1973a: 78 [incorrect subsequent spelling].

Type Locality: “Laguna Yema, Formosa”.

Type Material: paratypes MLP 735 (2); IFML 735 (2).

Distribution: Argentina, *Chaco Prov.*, Almirante Brown Dept., Taco Pozo (IFML 166). *Formosa Prov.* (MLP 11002, MLP 10999, MLP 11004, MLP 11003, MLP 11001, MLP 11000). *Tucuman Prov.*, Leales Dept., Estación Araoz (IFML 12860).

Remarks

The name *elatior* is the eugraph (“correct spelling”) of the name (Art. 32.2 of the Code).

Bulimulus fourmiersi
(d’Orbigny, 1835)
[new combination]

Bulimus fourmiersi d’Orbigny, 1835: 273; – d’Orbigny, 1837 [1834–1847]: pl. 30, figs. 12–14; 1838: 273; Pilsbry, 1897: 71, pl. 11, figs. 26–28.

Bulimulus fourmiersi – Parodiz, 1946: 315.
Drymaeus fourmiersi – Fernández, 1973a: 113.
Thaumastus fourmiersi – Doering, 1879: 73.
 ?*Naesiotus fourmiersi* – Breure, 1979: 136
 [nomina inquirenda].

Type Locality: "...province de Corrientes, non loin du Rio de Santa-Lucia, au lieu nommé *Pasto reito*...".

Type Material: Not located.

Distribution: Argentina, *Entre Rios Prov.*, Sierra de Cordona (MACN-In 29941). *Santa Fe Prov.*, San Nicolás (MACN-In 6433). *Misiones Prov.*, Guaraní Dept., El Soberbio (IFML 910), Iguazú Dept. (MLP 8063).

Bulimulus gracilis
 Hylton Scott, 1948
 [new status]

Bulimulus sporadicus gracilis Hylton Scott, 1948a: 238.

Bulimulus bonariensis gracilis – Parodiz, 1957b: 133.

Bulimulus (Bulimulus) bonariensis gracilis – Fernández, 1973a: 77.

Bostryx tortoranus Miquel, 1993: 123 [partim].

Type Locality: no mentioned by the author in the original description. The original label of the type material says: "Cerro Colorado, S. E. Salta".

Type Material: holotype MLP 11015, paratypes MLP 11014 (4).

Distribution: Argentina, *Salta Prov.*, Metán Dept., Cerro Colorado (IFML 15655).

Remarks

B. gracilis was established as a subspecies of *Bulimulus sporadicus* by Hylton Scott. However, it is here considered as a distinct species by its shell characters and protoconch sculpture, which differ from *B. sporadicus* (d'Orbigny). In *B. gracilis*, shell surface is covered with wrinkles; protoconch sculpture with axial wrinkles, of regular thickness, spaced, slightly wavy at middle portion, crossed by spiral lines, very faint tight layout. Shell aperture is oval, longer than wide. Shell size in general larger than *B. sporadicus*.

Bulimulus prosopidis
 Holmberg, 1912
 Fig. 1M

Bulimulus (Mesembrinus) prosopidis Holmberg, 1912b: 148, figs. 3, 4.

Bulimulus (Bulimulus) prosopidis – Parodiz, 1946b: 315; 1957a: 134; Fernández, 1973a: 80; Quintana, 1982: 90.

Bulimulus prosopidis – Breure, 1978: 147, figs. 237–238, pl.7, figs. 14, 15, pl. 16, fig. 1; 1979: 62; Miquel, 1991: 103, figs. 1, 2.

Type Locality: "Ad marginem dextrum fluminis Pilcomayo...". The Pilcomayo River runs through the limits between Argentina (Formosa Prov.) and Paraguay, and as Holmberg's (1912b) publication is referred to Argentinean Bulimulinae, we sustain that the type locality of *Bulimulus prosopidis* is in Argentina. Holmberg also mentioned that this species is found in Bolivia in a similar environment.

Type Material: lectotype MACN-In1309, paralectotypes MACN-In1309-1 (2) [present designation]. Lectotype: H = 24.71, DM = 8.93, Hap = 9.80, Dap = 5.78.

Distribution: Paraguay; Bolivia; Argentina, *Formosa Prov.*, Pilcomayo River (MACN-In 1309, MACN-In 1310).

Bulimulus rushii
 Pilsbry, 1897

Bulimulus rushii Pilsbry, 1897a: 18; – Formica Corsi, 1900: 409; Parodiz, 1962: 433; Miquel, 1991: 103, figs. 3–4; Breure, 1979: 62; Scarbino, 2003: 208.

Bulimulus (Bulimulus) rushii – Pilsbry, 1897: 70, pl. 12 fig. 47; Parodiz, 1944: 3; 1946b: 306, pl. 1, fig. 3; Fernández, 1973a: 80.

Peronaeus (Lissoacme) curamalalensis Parodiz, 1957b: 23.

Type Locality: "Maldonado, Uruguay".

Type Material: lectotype ANSP 70382.

Distribution: Uruguay; Argentina, *Buenos Aires Prov.*, La Plata (MACN-In 29938, 29939). *Corrientes Prov.*, Sauce Dept. (MLP 8087, 8088, MACN-In 37036). *Entre Rios Prov.* (MACN-In 9898), Colón Dept. (MACN-In 29937); *Paraná Dept.* (MLP 1311, 12956).

Remarks

Part of the material collected in Entre Ríos (MACN-In 12956) is labeled as *Bulimulus durus* Spix, 1847, a species similar in shell morphology and protoconch sculpture to *B. rushii*. Simone (2006) classified *Bulimulus durus* in the genus *Rhinus* Albers, 1860. The mentioned lot at MACN-In is classified here as *B. rushii*, but the future availability of soft material of *Bulimulus durus* and *B. rushii* will improve our knowledge providing a more consistent conclusion on the status of these two species.

Bulimulus vesicalis
(Pfeiffer, 1853)

Bulimus vesicalis Pfeiffer, 1853: 58; – Breure, 1978: 149.

Bulimus fayssianus Petit, 1853: 250, pl. 8, fig. 7.

Bulimulus (Bulimulus) vesicalis vesicalis – Pilsbry, 1897: 69, pl. 12, fig. 42.

Bulimulus (Bulimulus) vesicalis – Parodiz, 1946b: 315.

Peronaeus (Lissoacme) fayssianus – Breure, 1975: 1141.

Discoleus fayssianus – Breure, 1979: 91.

Bulimulus vesicalis vesicalis – Miquel, 1991: 105, fig. 6.

Type Locality: “Brasilia”.

Type Material: lectotype NHMUK 1975395; paralectotypes NHMUK 1975396 (2).

Distribution: Brazil; Uruguay; Argentina, *Buenos Aires Prov.*, Punta Lara (MACN-In 37176). *Entre Rios Prov.*, Paraná Dept. (MLP 8183).

Remarks

According to Miquel (1991) this species is also distributed in Santa Fé Prov., but we were unable to find any lot of this species at main Argentinean museums. Both, Parodiz (1957a) and Fernández (1973) cited that *Bulimulus vesicalis uruguayanus* Pilsbry, 1897, is present in Argentina as a Pleistocene subfossil.

Genus *Drymaeus*
Albers, 1850

Type Species: *Helix hygrophylaea* d’Orbigny, 1835 [subsequent designation by Pilsbry, 1896–1897].

Geographic Distribution: Argentina, Bolivia, Uruguay, Brazil, Paraguay, Peru, Ecuador, Colombia, Venezuela, Panama, Costa Rica, Nicaragua, Honduras, Guatemala, Mexico.

Remarks

Drymaeus, according to Pilsbry, includes most species referred to *Mesembrinus*, *Eudiotus*, *Mormus*, *Hamadryas* and *Liostracus* by Albers (1850). Several subgenera have been proposed for *Drymaeus*, from which Argentinean species are usually classified into *Drymaeus (Drymaeus)* and *Drymaeus (Mesembrinus)*. However, according to Pilsbry (1901) “the passage of *Drymaeus* with expanded or reflexed peristome to *Mesembrinus* with simple and acute, is a gradual one, and many species are arbitrarily assigned to one or the other subgenus”. Moreover, he considered that *Mesembrinus* is not a natural group and this name should not be used. Breure (1979) listed the species composition for each subgenus and provided subgenera diagnoses that are not based on apomorphies. For this reason, species listed will not be classified into subgenera awaiting a genus revision to test the validity of subgeneric divisions.

Drymaeus abyssorum
(d’Orbigny, 1835)

Helix abyssorum d’Orbigny, 1835: 17; – d’Orbigny, 1837 [1834–1847]: pl. 39, figs. 7, 8; 1838: 308; Miquel, 1989: 79, fig. 1.

Bulimus (Bulimulus) abyssorum – Beck, 1837: 64.

Bulimus (Scutalus) abyssorum – Albers, 1850: 161.

Drymaeus (Drymaeus) abyssorum – Pilsbry, 1898: 192, pl. 37, figs. 3, 4; Parodiz, 1957b: 24; 1962: 439; Fernández, 1973a: 113.

Drymaeus (Drymaeus) abyssorum – Breure, 1979: 106.

Drymaeus abyssorum – Breure, 1975: 1149, pl. 7, fig. 2; Richardson, 1995: 95.

Type Locality: “provincia Lagunacensi (republica Boliviana)”. In 1837 (1834–1847), d’Orbigny added: “sur les rives du Rio grande, entre Valle Grande et les Pescado ... principalement au lieu nommé Pampa Ruiz”.

Type Material: lectotype MNHN 24660; paralectotypes MNHN 24661(4); NHMUK 1854.12.4.125 (5).

Distribution: Bolivia; Brazil; Argentina, *Jujuy Prov.*, Dr. Manuel Belgrano Dept., Lozano (IFML 15660), Yala (IFML 15708). *Salta Prov.*, Corniza road from Salta to Jujuy (IFML 537).

Remarks

Miquel (1989b) synonymized *D. abyssorum* (d'Orbigny, 1835) with *D. hygrophylaeus* (d'Orbigny, 1835). Here, we consider that they are different species, in concordance with d'Orbigny. *Drymaeus abyssorum* is more elongate, with a smaller shell diameter and a less expanded peristome. Animal coloration is different, mostly yellowish in *D. abyssorum* and generally reddish in *D. hygrophylaeus*. In Argentina, *D. abyssorum* inhabits mostly cloud forest in Jujuy western areas, whereas *D. hygrophylaeus* is currently restricted to "El Rey" National Park in Salta and to a small patch of cloud forest in a road between Salta and Jujuy provinces.

Drymaeus flossdorfi
(Holmberg, 1909)
Fig. 1N
[new combination]

Bulimulus Flossdorfi Holmberg, 1909a: 11; – Parodiz, 1957a: 134; Fernández, 1973a: 79. *?Bostryx flossdorfi* – Breure, 1979: 136 [*nomina inquirenda*].

Type Locality: "(Territorio de Formosa), prope Nueva Pompeya".

Type Material: lectotype MACN-In 1352, paralectotype MACN-In 1352-1 (1) [present designation]. Lectotype: H = 30.73, DM = 12.77, Hap = 13.49, Dap = 7.77; paralectotype: H = 23.6, DM = 11.65, Hap = 11.79, Dap = 7.68.

Distribution: Paraguay?; Argentina, *Formosa Prov.*, Nueva Pompeya (MACN-In 1352). *Salta Prov.*, Rosario de la Frontera Dept. (MACN-In 1327). *Santiago del Estero Prov.*, Alberdi Dept., Campo Gallo (MACN-In 30616).

Remarks

MACN-In material from which the lectotype was selected was obtained from Holmberg's collection, originates from the type locality and shows same shell measurements. For this reason, we consider this material as syntypes.

On the other hand, according to Parodiz (1946a), this species is possible a synonym of *B. sporadicus*. Miquel (1991) assigned *B. flossdorfi* to *Bulimulus bonaeriensis sporadicus*. We tentatively classified *B. flossdorfi* to *Drymaeus*, because it presents the characteristic protoconch sculpture of a *Drymaeus* species. However, it would be necessary to collect fresh material that allows studying the anatomy to provide more supporting evidence.

Drymaeus hyltoni
Parodiz, 1957b

Drymaeus alabastrinus Hylton Scott, 1952: 25, non Da Costa, 1906; – Miquel, 1989b: 79, fig. 5.

Drymaeus hyltoni Parodiz, 1957b: 25; – Fernández, 1973a: 114; Richardson, 1995: 137.

Drymaeus (Drymaeus) hyltoni – Breure, 1979: 110; Miquel, 1989b: 78, figs. 18, 19.

Drymaeus alabastrinus – Breure, 1979: 106.

Type Locality: "Tartagal, Salta". This locality is located in General José de San Martín Dept.

Type Material: paratype MACN-In 32786.

Distribution: Argentina, *Jujuy Prov.*, *Salta Prov.*, General San Martín Dept., Tartagal (MACN-In 32786, MLP 11106).

Remarks

Hylton Scott originally named this species *D. alabastrinus*, and Parodiz (1957) recognized that it was pre-occupied by Da Costa (1906) for *D. alabastrinus* from Colombia. The type material of *D. hyltoni* is therefore the lot of *D. alabastrinus* housed at MACN-In collection on which Hylton Scott based the description of the species.

Drymaeus hygrophylaeus
(d'Orbigny, 1835)

Helix hygrophylaea d'Orbigny, 1835: 18.

Bulimus (Bulimulus) hygrophylaeus – Beck, 1837: 63.

Bulimus hygrophylaeus – d'Orbigny, 1837 [1834–1847]: pl. 40, figs. 3–5; 1838: 311.

Bulimus Drymaeus hygrophylaeus – Albers, 1850: 156.

Drymaeus (Drymaeus) hygrophylaeus – Pilsbry, 1898: 194, pl. 37, figs. 9–11; Breure &

Eskens, 1981: 28, figs. 77–83, pl. 1, fig. 3; Miquel, 1989b: 77, 79, figs. 3, 4.

Drymaeus hygrophylaeus – Parodiz, 1946b: 315, pl. 1, fig. 7; 1957b: 24; Fernández, 1973a: 113; Breure, 1975: 1151; Quintana, 1982: 97; Richardson, 1995: 136.

Drymaeus harringtoni Marshall, 1931: 2; – Miquel, 1989b: 79, fig. 2.

Type Locality: “provincia Chiquitensi (republica Boliviana)”. In 1837 (1834–1847), d’Orbigny added: “nous avons rencontré cette belle espèce dans les forêts chaudes et humides des parties orientales de la province de Santa-Cruz de la Sierra (Bolivia), Dans toutes les forêts au nord de Chiquitos ...”.

Type Material: syntypes MNHN 24670 (1), Bolivia, Guarayos; MNHN 24669 (1) Bolivia, Yungas, Guarayos; MNHN 24668 (3) Bolivia, Monte Grande, Chiquitos; NHMUK1854.12.4.126-128 (12).

D. harringtoni: holotype: USNM 202509.

Distribution: Bolivia; Paraguay; Argentina, *Jujuy Prov.* (MLP 8561), Dr. Manuel Belgrano Dept., Guerrero (MACN-In 16232), Las Capillas (IFML 643, MLP 8562); San Pedro Dept., La Mendieta (MLP 85609); Santa Bárbara Dept., Finca Portal del cerro (IFML 14733); Valle Grande Dept., Valle Grande (IFML 14452). *Salta Prov.*, camino de corniza (MACN-In 33435, IFML 961), Río Pescado (IFML 1497); Anta Dept., El Rey National Park (IFML 15699 A); La Caldera Dept., La Caldera (IFML 985); Orán Dept., Orán (MACN-In 20472).

Drymaeus interpunctus
(Martens, 1887)

Bulimulus interpunctus Martens, 1887: 161.

Drymaeus interpunctus – Pilsbry, 1898: 287, pl. 5, figs. 4, 5; Parodiz, 1957b: 25; 1962: 437; Fernández, 1973a: 114; Quintana, 1982: 95.

Drymaeus (Mesembrinus) interpunctus – Breure & Eskens, 1981: 74, figs. 235–244; Miquel, 1989b: 81, figs. 13, 16.

Type Locality: “interpunctus, Piracicaba, Nehring”, in the handwriting of Martens. Piracicaba is located in Brazil, San Pablo state.

Type Material: lectotype ZMB 38952a; paralectotypes ZMB 38952b (5).

Distribution: Uruguay; Brazil; Paraguay; Argentina, *Misiones Prov.*, Candelaria Dept., Cerro

Corá (MLP 8563); Capital Dept., Posadas (MLP 8565); El Dorado Dept., El Dorado (MLP 8567); Guaraní Dept., El Soberbio (IFML 11218); Iguazú Dept., Cataratas del Iguazú (MACN-In 27289, MLP 8569).

**Drymaeus lynchi*
Parodiz, 1946
Fig. 10

Drymaeus lynchi Parodiz, 1946a: 1; – Parodiz, 1946b: 316, pl. 2, fig. 3; 1957b: 25; 1962: 439; Fernández, 1973a: 115.

Type Locality: “Bolivia, Pozo de Vargas”. In 1962, Parodiz added that “Pozo Vargas, is in the zone of Oriental Cordillera of Bolivia between Rio Grande and Parapeti, is probably an old toponym no longer in use, inasmuch as it is not found in modern maps”. Parodiz (1962) also stated that *Drymaeus lynchi* was found at Corumbá, on the right shore of Paraguay River in the Brazil-Bolivian border.

Type Material: holotype MACN-In 1344; paratypes MACN-In 1344-2 (5).

Distribution: Bolivia; Paraguay; Brazil; Argentina, *Corrientes Prov.*

Remarks

Holotype and paratypes are the unique material existing in the main Argentinean malacological collections examined. Parodiz (1957b) cited this species as inhabiting the Argentinean border with Bolivia. Fernández (1973a) mentioned that distribution of *D. lynchi* is in north-eastern Argentina and southern Brazil.

**Drymaeus oreades*
(d’Orbigny, 1835)

Helix oreades d’Orbigny, 1835: 11.

Bulimus oreades – d’Orbigny, 1837 [1834–1847]: pl. 31, figs. 11, 12; 1838: 270.

Bulimus (Mesembrinus) oreades – Albers, 1850: 158.

Thaumastus oreades – Doering, 1879: 73.

Drymaeus oreades – Pilsbry, 1898: 277, pl. 44, figs. 95, 96; Parodiz, 1957b: 25; 1962: 439; Fernández, 1973a: 115; Richardson, 1995: 158.

Type Locality: “provincia Corrientes (republica Argentina)”. In 1837 (1834–1847), d’Orbigny added: “Nous l’avons rencontré dans un bois

humide de la rive sud Rio de Santa-Lucia dans les environs de San Roque, province de Corrinetes (république Argentine)".

Type Material: syntypes NHMUK1854.12.4.161 (6).

Distribution: Brazil; Paraguay; Argentina, *Corrientes Prov.*?

Remarks

Parodiz (1962) stated that *D. oreades* had not been collected in Corrientes since the times of d'Orbigny and that it is more abundant in southern Brazil. Therefore, the presence of *D. oreades* in Argentina needs to be proved.

Drymaeus papyraceus (Mawe, 1823)

Helix papyracea Mawe, 1823: 168.

Bulimus papyraceus – Reeve, 1849: pl. 39, fig. 236; Parravivini, 1894: 6; Ancey, 1897: 5.

Bulimus (Bulimulus) papyraceus – Beck, 1837: 65.

Bulimus (Mesembrinus) papyraceus – Albers, 1850: 157.

Helix lita var. *major* d'Orbigny, 1835: 11.

Bulimus lita d'Orbigny, 1838 [1834–1847]: 268.

Otostomus (Mormus) Papyraceus – Doering, 1879: 77 [incorrect subsequent spelling].

Drymaeus (Drymaeus) papyraceus – Pilsbry, 1898: 250, pl. 51, figs. 1–3; Breure & Eskens, 1981: 32, figs. 100, 101.

Drymaeus papyraceus – Parodiz, 1946b: 358; 1957b: 24; 1962: 444; Fernández, 1973a: 115; Quintana, 1982: 97; Richardson, 1995: 159.

Drymaeus papyraceus var. *papyrifactus* – Pilsbry, 1898: 252, pl. 51, figs. 4, 5.

Drymaeus papyraceus papyrifactus – Parodiz, 1957b: 24; 1962: 444; Fernández, 1973a: 116; Miquel, 1989b: 78, figs. 6–8, 17; Scarbino, 2003: 209.

Type Locality: In the original description the type locality was not specified. Later, Ancey (1897) stated "...elle [*D. papyraceus*] vit dans la region de Corrientes". Pilsbry (1898) stated that "The type locality of *papyraceus* is Rio de Janeiro".

Type Material: Not located.

Distribution: Brazil; Uruguay; Paraguay; Argentina, *Buenos Aires Prov.*, Martín García Island

(MACN-In 22868, MACN-In 17702, MACN-In 11961, MACN-In 21051, MLP 8579). *Corrientes Prov.* (MACN-In 944), Capital Dept., Riachuelo (MACN-In 17479); Ituzaingó Dept., Puerto Luján (MACN-In 561); San Martín Dept., Laguna del Iberá (MACN-In 30663). *Formosa Prov.*, Laishi Dept., El Bagual (IFML 14358). *Misiones Prov.*, Puerto Bemberg (IFML 231), Concepción Dept., Santa María (MACN-In 8240); El Dorado Dept., El Dorado (MLP 8576, MLP 8577).

Remarks

D'Orbigny (1838 [1834–1847]) recognized two distinct varieties of this species. Later, Pilsbry (1898) explained that typical *papyraceus* is always elongate and narrow and inhabits in littoral of Brazil, from Rio Grande do Sul Province towards Bahia and Pernambuco states. The variety *papyrifactus*, is always shorter with more open aperture, living in Corrientes and Misiones provinces, Argentina.

Drymaeus poecilus (d'Orbigny, 1835)

Helix poecila d'Orbigny, 1835: 11.

Bulimus poecilus var. *major* d'Orbigny, 1837 [1834–1847]: pl. 31, figs. 1–10; 1838: 268.

Bulimus (Mesembrinus) poecilus – Albers, 1850: 158.

Otostomus (Mesembrinus) poecilus – Doering, 1879: 76.

Bulimulus poecilus – Ancey, 1892: 92.

Drymaeus poecilus – Pilsbry, 1898: 285, pl. 49, figs. 49–57; Parodiz, 1946b: 316, 321, fig. 7; 1957b: 25; 1962: 440; Fernández, 1973a: 116; Breure, 1975: 1152; Richardson, 1995: 163.

Drymaeus (Drymaeus) poecilus – Breure & Eskens, 1981: 32, figs. 102–108; Miquel, 1989b: 80, figs. 9–11, 14, 15.

Drymaeus (Drymaeus) poecilus poecilus – Parodiz, 1962: 441; Fernández, 1973a: 117.

Drymaeus (Drymaeus) poecilus minor – Parodiz, 1957b: 25; 1962: 441; Fernández, 1973a: 117.

Drymaeus poecilus poecilus – Quintana, 1982: 95.

Type Locality: "provincia Chiquitensi (republic Argentina)". In 1837 (1834–1847), d'Orbigny added the following details: "La varieté *major* habite les vallées des derniers contreforts des Andes boliviennes, avant de descendre dans les plaines de Santa-Cruz

de la Sierra, surtout à la porte de Tasajos et du bourg de Pampa grande..." "La variété *minor*...est propre aux immenses forêts qui bordent le Rio grande, entre Santa-Cruz de la Sierra et Chiquitos;...d'autres sous-variétés sont confinées à deux lieux plus à l'est Dans les forêts qui bordent le Rio de Tucabaca, entre San-Juan et Santo Corazon de Chiquitos".

Type Material: syntypes MNHN 24679; MNHN 24678, Bolivia, Pampa Grande; MNHN 24677, Bolivia, Chiquitos; MNHN 24676, Bolivia, Chiquitos; MNHN 24675, Bolivia, San Juan, Pampa Grande; NHMUK1854.12.4.156-157 (18) Bolivia, Chiquitos.

Distribution: Bolivia; Paraguay; Argentina, *Catamarca Prov.*, Sierra del Alto (MACN-In 25871); Andalgalá Dept., Choya (MACN-In 25742); Belén Dept., Aguas de Dionisio (IFML 291); Paclín Dept., La Viña (MLP 8594). *Formosa Prov.* (MLP 8586), Bermejo Dept., Laguna Yema (MLP 8592); Matacos Dept. (IFML 15676). *Jujuy Prov.*, Obrajo, Palo Santo (IFML 11222), between Chalica y Quemado (IFML 7), on the road between Jujuy and San Pedro (IFML 593), 6 Km South Embarcación, Bermejo river (IFML 15664), South of Urundel (IFML 902); El Carmen Dept., Puesto Viejo (MACN-In 985, MLP 986); Ledesma Dept., Calilegua National Park (IFML 14181, IFML 14181), Ledesma (MLP 2952); San Pedro Dept. (MLP 8585); Santa Bárbara Dept. (MLP 8593). *Salta Prov.* (IFML 12765, IFML 15674), between Lumbrera and Juramento (IFML 576, IFML 811), Pileta (IFML 15669), 12 Km North of Yuto (IFML 15673), 50 km from Salta (IFML 12765), on the road between Salta and Cafayate (IFML 583); Anta Dept. (IFML 6134), Las Víboras (MLP 8589); Cafayate Dept., Quebrada de Las Conchas (MLP 2359); Capital Dept., Cerro San Bernardo (IFML 15662); Cerrillos Dept., Cerrillos (IFML 15695 A, IFML 15661); General Güemes Dept., Las Mesitas (IFML 15670); General José de San Martín Dept., Embarcación (MACN-In 8848-4, IFML 1404), Pocitos (MLP 8584), Tartagal (IFML 24); La Viña Dept., Alemania (MACN-In 585, MACN-In 17592, MLP 17592, MLP 2370, MLP 8587, MLP 2427), Talapampa (MLP 8588); La Candelaria Dept., pasando el Espinal (IFML 15688); Metán Dept., Cerro Colorado (MACN-In 26520, MLP 26520, IFML 828, IFML 15758, IFML 804), Dique Cabra Corral (IFML 15689), El Galpón (IFML 926), Lumbreras (MACN-In 23047, MLP 23047,

MLP 8590, IFML 811, IFML 420), Juramento River (MLP 19393, IFML 810, IFML 140), on the road from Metán to Güemes (IFML 1523, IFML 15672), on the road from Metán to El Galpón (IFML 15674); Orán Dept., Orán (MLP 8582), 8 Km N of Urundel (IFML 15668, IFML 11298), Urundel (IFML 15671, IFML 163, IFML 902, IFML 11298, MLP 8580), Manuel Elordi (MLP 8581); Rosario de la Frontera Dept. (MACN-In 6185, MLP 2776, IFML 2128, IFML 15665). San Juan Prov., Valle Fértil Dept., Ischigualasto (IFML 504). *Santiago del Estero Prov.*, Sierra de Guasayan (MACN-In 12950, MACN-In 13972, MLP 25848, IFML 14899), Pellegrini Dept., Cerro del Remate (IFML 209). *Tucuman Prov.* (IFML 11307, IFML 15666), on the road from Tucuman to La Cocha (IFML 15679), San Ignacio (IFML 1401); Burruyacu Dept. (IFML 11300, IFML 14900), El Duraznito (IFML 129); Capital Dept., San Miguel de Tucuman (IFML 15697A, IFML 14256); La Cocha Dept., Dique San Ignacio (IFML 964); Leales Dept., Estación Araoz (MACN-In 18318, MLP 18318, IFML 269); Juan Bautista Alberdi Dept., Escaba (IFML 15678); Leales Dept., El Guardamonte (IFML 146); Monteros Dept., Huasa Pampa (IFML 867); Tafí Viejo Dept., El Cadillal (MACN-In 9008, MLP 9008, IFML 151, IFML 14265), Los Nogales (IFML 81), San Javier National Park (IFML 14985); Trancas Dept., between Tapia and Vipos (IFML 10836, IFML 15667, IFML 15675, IFML 15677), El Boyero (IFML 14767), on the road from Tapia to Raco (IFML 853, IFML 15663), San Pedro de Colalao (MACN-In 9860-2, IFML 10839, IFML 15680), Tapia (IFML 853, IFML 10994, IFML 10836, IFML 10994, IFML 15683), Ticucho (IFML 14901), Vipos (MACN-In 17579, MACN-In 17593, IFML 15696 A, MLP 8595, IFML 11295, IFML 15682).

Remarks

D'Orbigny (1837, 1838 [1834–1847]) recognized two varieties of *Drymaeus poecilus*, variety *major* inhabiting the valleys of easternmost foothills of Bolivian Andes to the plains of Santa Cruz de la Sierra, and variety *minor* inhabiting forests along Rio Grande between Santa Cruz de la Sierra and Chiquitos in Bolivia. A third variety, *ictericus*, was introduced by Ancy (1892) in Matto Grosso, Brazil, based on two dry shells. In Argentina, the three varieties have been previously cited by Parodiz (1957b) and Fernández (1973) in their catalogues for Argentinean mollusks. However, as previously

stated by Parodiz (1962), d'Orbigny defined *D. poecilus major* as the first, more abundant form, and it is considered to be the nominal subspecies. There are no substantial differences between *D. poecilus major* and *D. poecilus minor* except for their shell sizes, for this reason they should not be treated as different entities, following Miquel's (1989b) criteria. *Drymaeus poecilus ictericus* has not been collected in any locality of Argentina and therefore is not mentioned in the present catalogue.

Genus *Naesiotus*
Albers, 1850

Type Species: *Bulimus nux* Broderip, 1832 [subsequent designation by Dall, 1896].

Geographic Distribution: Argentina, Bolivia, Brazil, Uruguay, Paraguay, Perú, Ecuador (including Galápagos), Colombia, Venezuela, Lesser Antilles.

Naesiotus calchaquinus
(Doering, 1879)

Bulimulus (Peronaeus) calchaquinus Doering, 1879: 64.

Bulimulus (Bostryx-Peronaeus) calchaquinus – Pilsbry, 1896: 151.

Peronaeus (Peronaeus) calchaquinus – Parodiz, 1946b: 315; Fernández, 1973a: 87.

Peronaeus calchaquinus – Parodiz, 1957b: 22.

Bostryx calchaquinus – Breure, 1979: 52.

Naesiotus calchaquinus – Miquel, 1989a: 63, figs. 1–3.

Type Locality: "Sierra de Belén". The mountain system called Sierra de Belén is located in Catamarca Prov., Belén Dept.

Type Material: Not located.

Distribution: Argentina, Catamarca Prov., Ambato Dept., La Puerta (MACN-In 483); Capayán Dept. (MACN-In 17775).

Naesiotus crepundia
(d'Orbigny, 1835)

Helix (Cochlogena) crepundia d'Orbigny, 1835: 14.

Bulimulus crepundia – d'Orbigny, 1837 [1834–1847]: pl. 33, figs. 18, 19; 1838: 275; Zischka, 1953: 78.

Bulimus rivasii d'Orbigny, 1837 [1834–1847]: pl. 34, figs. 8, 10; 1838: 276 – Pfeiffer, 1848: 155; Reeve, 1849: 20, pl., 122, fig.; Gray, 1854: 16.

Bulimulus rivasii – Zischka, 1953: 80.

Bulimulus crepundia – Reeve, 1849: 47, fig. 330

Bulimulus (Protoglyptus) crepundia – Pilsbry, 1897: 90, pl. 11, figs. 33, 34.

Bulimulus (Protoglyptus) rivasii – Pilsbry, 1897: 91, pl. 12, figs. 40, 41.

Protoglyptus punctustriatus Parodiz, 1946a: 315; 1957b: 22.

Protoglyptus (Protoglyptus) punctustriatus – Parodiz, 1946b: 315, 352, pl. 1, fig. 5; Fernández, 1973a: 103.

Protoglyptus (Protoglyptus) crepundia – Parodiz, 1946b: 315, 319, fig. 4, pl. 1, fig. 11.

Protoglyptus (Protoglyptus) rivassi – Parodiz, 1946b: 315.

Naesiotus punctustriatus – Breure, 1979: 71.

Naesiotus rivasii – Breure, 1975: 1147; Miquel, 1989a: 70, figs. 12–14; Ramírez et al., 2003: 280.

Naesiotus crepundia – Breure, 1975: 1146, pl. 8, fig. 6; 1979: 68.

Type Locality: "provincia Chiquitensi (republica Boliviana)". In 1837 (1834–1847) d'Orbigny added "... sur les coteaux de gres ancien du Sutos, pres de la Mission de San José".

Type Material: lectotype MNHN 25403, paralectotypes (3) MNHN 25404.

Protoglyptus (Protoglyptus) punctustriatus: holotype MACN-In 991; paratypes MACN-In 991-1 (4).

Distribution: Bolivia; Brazil; Peru; Argentina, Jujuy Prov., Santa Bárbara Dept., Puesto Viejo (MACN-In 991, MACN-In 991-1). Salta Prov., Anta Dept. (MACN-In 367); General José de San Martín Dept., Embarcación (MACN-In 8848, FMNH 125187, FMNH 30613).

Naesiotus deletangi
(Parodiz, 1946)

Protoglyptus deletangi Parodiz, 1946a: 3.

Protoglyptus (Rimatula) deletangi – Parodiz, 1946b: 315, pl. 1, fig. 6; Fernández, 1973a: 103.

Protoglyptus (Rimatula) minutissimus Parodiz, 1962: 445.

Naesiotus deletangi – Breure, 1979: 69; Miquel, 1989a: 65, figs. 5, 6.

Type Locality: “Embarcación, provincia de Salta, Republica Argentina”. This locality is located into General José de San Martín Dept., Salta Prov.

Type Material: holotype MACN-In 3131; paratypes MACN-In 3131-1 (17).

Distribution: Bolivia; Argentina, *Salta Prov.* (FMNH 125935, FMNH 30614), General José de San Martín Dept., Embarcación (MACN-In 3131, MACN-In 3131-1), Las Tablillas (MACN-In s/n), General Mosconi and Estación Vespucio (MLP s/n); Orán Dept., Urundel (IFML 1497). *Tucuman Prov.* (IFML 128), Burruyacú Dept., El Duraznito (IFML 127), San Pedro de Colalao (IFML 816, IFML 4014); Juan Bautista Alberdi Dept., Escaba (IFML 14247); La Cocha Dept., Dique San Ignacio (IFML 971); Tafí Viejo Dept., San Javier National Park (IFML 14982, IFML 14983, IFML 14984); Trancas Dept. (IFML 15250), El Cadillal (IFML 14272, IFML 15179, IFML 15180, IFML 15181).

Naesiotus montivagus
(d’Orbigny, 1835)

Helix (Cochlogene) montivaga d’Orbigny, 1835: 14.

Bulimus montivagus – d’Orbigny, 1837 [1834–1847]: pl. 34, figs. 1–3; 1838: 275; Pfeiffer, 1848: 112; Reeve, 1849: pl. 67, fig. 475; Gray, 1854: 15; Doering, 1874a: 54.

Peronaeus montivagus – Albers, 1850: 163.

Chondrus (Peronaeus) montivagus – Adams & Adams, 1858 [1853–1858]: 165.

Bulimus montivagus var. *chacoensis* Ancey, 1897: 16; – Breure, 2011: 19.

Bulimulus (Protoglyptus) montivagus – Pilsbry, 1897: 90, pl. 11, figs. 29, 30; pl. 14, figs. 14, 15.

Bulimulus montivagus – Pilsbry, 1901: 147.

Protoglyptus (Rimatula) montivagus – Parodiz, 1946b: 315, pl. 1, fig. 10; 1957b: 22; Fernández, 1973a: 103.

Naesiotus montivagus – Breure, 1975: 1146; Breure & Coppois, 1978: 178; Quintana, 1982: 94.

Naesiotus montivaga – Breure, 1979: 70 [incorrect subsequent spelling].

Type Locality: “provincia Lagunensi (republica Boliviana); et provincia Entre-Rios (republica Argentina)”. In d’Orbigny (1838 [1834–1847]) the author added to the information of the type locality: “ Nous avons rencontré cette espèce d’abord sur les coteaux du Parana ... province d’Entre-rios (république Argentine)

... des Andes orientales, dans les provinces de Valle Grande et de la Laguna, et dans le *Monte grande* ... qui sépare Santa –Cruz de la Sierra de la province de Chiquitos ...”.

Type Material: syntypes MNHN s/n: Bolivia (2); MNHN s/n Bolivia, Pampa (5).

Bulimus montivagus var. *chacoensis*: lectotype RBINS/MT 2342.

Distribution: Brazil; Bolivia; Paraguay; Argentina, *Catamarca Prov.* (MLP 1481), Ambato Dept., La Puerta (MACN-In 483), Las Trancas (MLP 1318). Entre Ríos Prov. (MACN-In 14644). *Salta Prov.*, Capital Dept., San Lorenzo (MACN-In 6587); General José de San Martín Dept., lomas near Tartagal (FMNH 30612), Vespucio (MACN-In 6511). *Jujuy Prov.* (IFML 856, IFML 15613). *Santiago del Estero Prov.*, Pellegrini Dept., Cerro del Remate (MACN-In 23045). *Tucuman Prov.*, Graneros Dept., La Madrid (MACN-In 12116); Trancas Dept., El Cadillal (IFML 15179, IFML 15180, IFML 15181).

Naesiotus munsterii
(d’Orbigny, 1837)

Bulimus munsterii d’Orbigny, 1837 [1834–1847]: pl. 34, figs. 4–7; 1838: 278; Pfeiffer, 1848: 109; Gray, 1854: 16.

Bulimulus (Bostryx-Lissoacme) munsteri – Pilsbry, 1896: 185, pl. 51, figs. 9–11 [incorrect subsequent spelling].

Bulimulus munsteri – Zischka, 1953: 79.

Protoglyptus (Protoglyptus) munsteri – Parodiz, 1946b: 315; Fernández, 1973a: 102.

Protoglyptus munsteri – Parodiz, 1957b: 22.

Protoglyptus ramosae Hylton Scott, 1951a: 23, pl. 1, fig. 6; – Parodiz, 1957b: 22.

Protoglyptus (Protoglyptus) ramosae – Fernández, 1973a: 101.

Naesiotus munsteri – Breure, 1975: 1147.

Naesiotus munsterii – Miquel, 1989a: 67, figs. 7, 8.

Naesiotus ramosae – Breure, 1979: 71.

Type Locality: “... la côte de *Petaca*, ... des Andes orientales ... les Plaines de Santa-Cruz de la Sierra, en Bolivia.”

Type Material: syntypes MNHN 25392 (2), Bolivie, Cote de la Petaca; probable syntypes MNHN 25393 (2), Bolivie, Chiquitos; MNHN 25394 (1), Bolivie, Rio Grande.

Protoglyptus ramosae: holotype MLP 11366; paratypes MLP 11365 (3), MLP 1137 (4).

Distribution: Bolivia; Argentina, *Salta Prov.*, General José de San Martín Dept., Embarcación (MACN-In 9341).

Naesiotus oxylabris
(Doering, 1879)

Bulimulus (Scutalus) oxylabris Doering, 1879: 65.

Bulimulus (Bulimulus) centralis Doering, 1879: 69.

Bulimulus (Bostryx-Lissoacme) centralis – Pilsbry, 1896: 188.

Bulimulus (Bostryx-Lissoacme) oxylabris – Pilsbry, 1896: 190.

Protoglyptus (Rimatula) oxylabris – Parodiz, 1946b: 315, 319, fig. 3, pl. 2, fig. 4; 1957b: 22; Fernández, 1973a: 104.

Naesiotus oxylabris – Breure & Coppo, 1978: 180; Miquel, 1989a: 68, figs. 10, 11.

Naesiotus centralis – Breure, 1979: 68.

Type Locality: “primera sierra de Cordoba, en los terrenos calcáreos como en la Calera, San Antonio Maldonado, Alta Gracia ...”. The original records indicated by Doering are located in Cordoba Prov.

Type Material: Not located.

Distribution: Chile; Argentina, *Cordoba Prov.* (ZMB 34720, FMNH 126456, MLP 9494, MACN-In 1882), Colón Dept., Calera (MACN-In 9949); Cruz del Eje Dept. (MACN-In 14857, MACN-In 9897, IFML 15630, MCZ 108687), Estancia La Fronda (IFML 15619), La Loma (MACN-In 1638), to San Marcos Sierra (IFML 15616); Ischilín Dept. (MLP 9495), between Ongamira and Ischilín (FMNH 36614), Deán Funes (IFML 10835), from Deán Funes to Sauce Punca (MACN-In 3238), Cerro San Vicente (IFML 15617), Copacabana (MLP s/n, FMNH 72241, FMNH 78341), Villa Mirador del Lago (IFML 15629); Pocho Dept., between Tanninga and Salsacate (IFML 10883, IFML 15631), Sierra de Pocho (MACN-In 920); Punilla Dept., Capilla del Monte (MACN-In 13255, MACN-In 1878, MACN-In 14855, IFML 830, IFML 15633), route to Capilla del Monte (IFML 15614, IFML 15615), Cerro Uriorco (MACN-In 37764), Cosquín (MACN-In 1651), Pampa de Olaen (MACN-In 1665), La Cumbre (MACN-In 9228, MACN-In 9226, MACN-In 3239, MACN-In 30572, MACN-In 1329), between La Cumbre and Capilla del Monte (MACN-In 9228), La Falda (MACN-In

1875, MACN-In 23555), Los Cocos (MACN-In 6581), Molinari (MCZ 263565), Ongamira (MLP 36009-2), San Antonio (MACN-In 10427), Valle Hermoso (MACN-In 1667, MACN-In 1628); Santa María Dept. (MACN-In 14856, MACN-In 291), Potrero de Garay (MACN-In 291); Totoral Dept. (FMNH 36613), Villa General Mitre (IFML 15632), La Loma (MACN-In 1638); Tulumba Dept., Tulumba (IFML 15618), route from Dean Funes to Tulumba (IFML 15656 A); Santa Rosa de Calamuchita Dept. (MACN-In 3221, MACN-In 23591), Calamuchita (MACN-In 3221), Est. Casa Grande (MACN-In 9697), Los Sauces (MLP 2388), Valle de los Reartes (MLP 1485, MACN-In 13253, MACN-In 9919, FMNH 53069, FMNH 31285, FMNH 126850, FMNH 17261), Villa General Belgrano (Embalse Los Molinos) (MCZ 239221). *San Luis Prov.*, General Pedernera Dept., Cerro del Morro (MACN-In 30575).

Naesiotus pollonerae
(Ancey, 1897)

Bulimulus pollonerae Ancey, 1897: 17; – Wood & Gallichan, 2008: 77.

Bulimulus (Protoglyptus) pollonerae – Pilsbry, 1901: 148, pl. 45, fig. 30.

Protoglyptus (Rimatula) pollonerae – Parodiz, 1946b: 315; 1957b: 22; Fernández, 1973a: 105.

Naesiotus pollonerae – Breure & Coppo, 1978: 181; Breure, 1979: 71; Miquel, 1989a: 69, fig. 17; Breure, 2011: 38.

Type Locality: “San Lorenzo, province de Jujuy, Republique Argentine”.

Type Material: lectotype RBINS/MT 2369(1); paralectotype RBINS/MT 2370(1).

Distribution: Argentina, ?*Buenos Aires Prov.*, hills of San Nicolás, margin of Parana river (MACN-In 6435); *Salta Prov.* (MACN-In 3132), Orán Dept., Urundel (IFML 15620).

Naesiotus rocyanus
(d’Orbigny, 1835)

Helix (Cochlogene) rocyana d’Orbigny, 1835: 13.

Bulimus rocyanus – d’Orbigny, 1837 [1834–1847]: pl. 33, figs. 6, 7; 1838: 277; Pfeiffer, 1848: 35.

“*Bulimulus*” *rocyanus* – Pilsbry, 1898: 321, pl. 44, figs. 4, 5.

Proto glyptus (*Obstrussus*) *rocayanus* – Parodiz, 1946b: 315; 1957b: 22; Fernández, 1973a: 105.

Drymaeus rocayanus – Zischka, 1953: 82.

Naesiotus rocayanus – Breure, 1975: 1147.

Naesiotus pollonerae – Miquel, 1989: 69 [partim].

Type Locality: “provincia Santa Cruz de la Sierra (republica Boliviana)”. In 1837 [1834–1847], d’Orbigny gave more details saying: “...bois épais qui bordent le Rio grande, au sud de Santa-Cruz de la Sierra, surtout près du hameau de *Pacu*”.

Type Material: lectotype MNHN 25391.

Distribution: Bolivia; Argentina, *Jujuy Prov.* (MACN-In 1341), *Ledesma Dept.*, *Calilegua National Park* (MLP s/n). *Salta Prov.*, *General José de San Martín Dept.*, *Carapará* (MACN-In 6513); *Guachipas Dept.*, *Alemanía* (MACN-In 17600).

Naesiotus willinki

Breure, 1978

Fig. 1P

Naesiotus willinki Breure, 1978: 162, figs. 262–269, pl. 12, fig. 2; – Breure, 1979: 72.

Naesiotus pollonerae – Miquel, 1989a: 69, figs. 18, 19 [partim].

Type Locality: “Argentina, Prov. Salta, Estancia Lumbrera, 750 m”.

Type Material: holotype IFML 1263a; paratypes IFML 1263 (11), IFML 815 (14), RMNH 55323 (10).

Distribution: Argentina, *Salta Prov.*, *Metán Dept.*, *Estancia Lumbrera* (IFML 1263a, IFML 815, RMNH 55323), 24 km from Metán on the road to Galpón (IFML 1264, RMNH 55324), between Lumbrera and Juramento (IFML 15707).

Genus *Scutalus*

Albers, 1850

Type Species: *Bulimus proteus* Broderip [subsequent designation by Albers, 1860].

Geographic Distribution: Argentina, Bolivia, Peru, Ecuador.

Scutalus tupacii
(d’Orbigny, 1835)

Helix Tupacii d’Orbigny, 1835: 16.

Bulimus tupacii – d’Orbigny, 1837 [1834–1847]: pl. 38, figs. 1, 5; 1838: 292; Pfeiffer, 1848: 52; Parravivini, 1894: 7.

Bulimulus (Scutalus) tupacii – Pilsbry, 1897: 19, pl. 3, figs. 27–31.

Bulimus (Orphus) tupacii major – Doering, 1875a [1877]: 338.

Bulimus (Orphus) tupacii minor – Doering, 1875a [1877]: 339.

Scutalus tupaci – Parodiz, 1946b: 315, 320, fig. 6, pl. 2, fig. 5; 1957a: 134; 1962: 450 [incorrect subsequent spelling].

Kuschelenia simulans Hylton Scott, 1951b: 540, figs. 1–4; – Fernández, 1973a: 87 [new synonymy].

Scutalus tupacii – Fernández, 1973a: 101; Breure, 1975: 1144, pl. 2, fig. 3.

Scutalus (Kuschelenia) tupacii – Breure, 1978: 186, fig. 136, pl. 18, figs. 7, 8; 1979: 88, fig. 140; Miquel, 1998: 183, figs. 8, 9, 14, 18, 19.

Type Locality: “Bolivia, La Paz, Yanacachi”.

Type Material: lectotype MNHN 24710, Bolivia; paralectotypes MNHN 24713(3), MNHN 24712 (4), MNHN 24711(2).

Kuschelenia simulans: holotype MLP 11219.

Distribution: Bolivia; Paraguay; Argentina, *Catamarca Prov.*, *Andalgalá Dept.*, *Las Estancias* (IFML 10940), *Sierra de Gracián* (IFML 257, IFML 15691); *Formosa Prov.*, *Pirané Dept.*, *Guayacan*, (IFML 236). *Jujuy Prov.*, *Ledesma Dept.*, *Calilegua National Park* (IFML 15559); *Palpalá Dept.* (MACN-In 8114); *Santa Barbara Dept.* (MACN-In 15948). *Salta Prov.*, *Anta Dept.*, *El Rey National Park* (IFML 14245); *Capital Dept.* (MACN-In 29965); *Rosario de Lerma Dept.* (MACN-In 10015). *Santiago del Estero Prov.*, *Capital Dept.*, *El Zanjón* (IFML 14277). *Tucuman Prov.*, *Burruyacu Dept.*, *Alto de Medina* (IFML 572), between Zamorá and Taficillo (IFML 624), from Acherál to Tafí del Valle (IFML 15684); *Capital Dept.* (MACN-In 35675, MACN-In 35676, MACN-In 6182); *Chicligasta Dept.*, *Alpachiri* (IFML 77), *Campo de Los Alisos National Park* (IFML 15247); *Lules Dept.*, *Villa Nougues* (MACN-In 1641, MACN-In 18320, IFML 10995, IFML 64, IFML 10493,

IFML 14243); Tafí del Valle Dept. (IFML 68, IFML 386), La Quebradita (IFML 15222), Quebrada Los Sosa (IFML 15690); Tafí Viejo Dept., El Cadillal (MACN-In 9007, MACN-In 9915, IFML 182, IFML 239, IFML 15221, IFML 14274, IFML 15224, IFML 15226, IFML 15686), Taficillo (IFML 11309, IFML 624); Yerba Buena Dept., Horco Molle (MACN-In 30612, IFML 452, IFML 569, 570, IFML 15692, IFML 14249, IFML 15223, IFML 15225), San Javier (MACN-In 1315, MACN-In 37821, IFML 14979, IFML 14580, IFML 14967, IFML 14980, IFML 14981, IFML 858, IFML 570, IFML 15693, IFML 15694); Trancas Dept., La Higuera (IFML 10942), Sierra del Nogalito (IFML 819, IFML 820, IFML 15687); Concepción Dept., Rio Cochuna (IFML 819).

Remarks

Breure (1975) stated that d'Orbigny figure of a specimen of *Scutalus tupacii* was designated lectotype because the three syntypes deposited at MNHN collection did not have exact localities. However, currently there is a specimen designated as "Lectotype" at MNHN collection, and its number is here provided.

Scutalus (Kuschelenia) simulans (Hylton Scott, 1951b) (Fig. 1Q) is here synonymized with *Scutalus tupacii* d'Orbigny, 1835, because the former species clearly falls between variable morphotypes described for *Scutalus tupacii*. These were illustrated by Pilsbry (1897). The smooth protoconch attributed to *S. (K.) simulans* by Hylton Scott in her original species description is a misinterpretation. The holotype, which is the single shell existing of *Scutalus (Kuschelenia) simulans*, has an eroded protoconch. However, typical sculpture of *S. tupacii* protoconch is visible in the surface close to suture between protoconch and first whorl in *S. (K.) simulans* holotype. There are no characters proper from *Scutalus (K.) simulans* that allows its differentiation from *S. tupacii*. The area of distribution of *S. (K.) simulans* is also included into the area of occurrence of *S. tupacii*.

Weyrauch identified as *Scutalus pluto* (Crosse) several lotes collected in Argentina, mainly in Tucumán and deposited at the malacological collection of IFML. However, we consider that these dry shells are also morphotypes that clearly falls into the ranges of variability proper of *Scutalus tupacii*. The lectotype of *S. pluto* at MNHN is also very similar in morphology and size to *S. tupacii*. Moreover, known area of occurrence of *Scutalus pluto* is beyond

Argentina limits, and this species is known to occur in Peru. *Scutalus pluto* does not occur in Argentina.

Recently, Breure (2011) restricted the genus *Scutalus* to the subgenus *Scutalus (Scutalus)* *sensu* Breure (1979), giving generic status to *Kuschelenia*. *Vermiculatus* was also considered by Breure (2011) a subgenus of *Kuschelenia*. Here, we followed the classification of *Scutalus* previously proposed by Breure (1979) because no clear taxonomic justification for the recent changes has been provided and therefore characters that allow a clear differentiation among these entities are lacking.

Family Odontostomidae
Pilsbry and Vanatta, 1898

Genus *Clessinia*
Doering, 1875

Type Species: *Clessinia stelzneri* Doering, 1875.

Geographic Distribution: Argentina.

Clessinia cordovana
(Pfeiffer, 1855)
[new combination]

Bulimus cordovanus Pfeiffer, 1855: 149; – Pfeiffer, 1856: 34; 1859: 435; Dohrn, 1875: 202; 1877: 157; Kobelt, 1878: 150; Martens, 1890–1891: 251; Breure, 1974b: 114.

Bulimus "Macrodonates" cordovanus – Doering, 1875a [1877]: 331.

Odontostomus (Scalarinella) cordovanus – Pilsbry, 1901: 66, pl. 13, fig. 100.

Odontostomus (Macrodonates) cordovanus – Holmberg, 1912b: 153.

Odontostomus (Scalarinella) cordovanus – Parodiz, 1939: 732, fig. 1.

Odontostomus (Scalarinella) cordovanus striatus – Parodiz, 1939: 733.

Cyclodontina (Scalarinella) cordovana – Parodiz, 1957b: 29.

Cyclodontina (Clessinia) cordovanus – Hylton Scott, 1966: 31, figs. 1–5, 7.

Cyclodontina (Clessinia) gracilis Hylton Scott, 1966: 34, fig. 6, 8 [new synonymy].

Clessinia cordovana – Fernández, 1973a: 142.

Clessinia gracilis – Fernández, 1973a: 144.

Odontostomus (Scalarinella) cordovanus striatus – Breure, 1974b: 124.

Cyclodontina (Clessinia) gracilis – Breure, 1974b: 116.

Type Locality: “Hab. In Andibus prope Cordova”. Doering (1875a [1877]) added that the type locality is found in Argentina, western slope of the *Sierra del Aconquija* in moist places, in Quebradas de la Mermela, de Jatan, del Nieve and further south close to Agua de los Oscuros.

Type Material: lectotype SMF 10417 a; paralectotypes SMF 10417 b, MLP 1493, MACN-In 9127.

Distribution: Argentina, *Catamarca Prov.*, Ambato Dept., Puerta de Ambato (MACN-In 421). *Cordoba Prov.* (FLMNH 109762, FLMNH 178615, MACN-In 9127), Cruz del Eje Dept., Serrezuela (MACN-In 19624); Ischilin Dept., Cerro San Vicente (IFML 10789, IFML 10739, MACN-In 9226); Pocho Dept., Sierra de Pocho (IFML 13521, IFML 15240, IFML 15241); Tulumba Dept. (FMNH 36596, IFML 14745), from San José de La Dormida to Tulumba (IFML 15245); Cruz del Eje Dept., San Marcos Sierra (IFML 15774 A, MACN-In 1593, IFML 15772 A, IFML 15770 A, IFML 15768 A).

Remarks

C. gracilis is here synonymized with *C. cordovana* because type general morphology and shell measurements falls into shell variability described for *Clessinia cordovana*. The area of distribution is also overlapping.

Clessinia stelzneri
(Doering, 1875)

Bulimus (Clessinia) stelzneri – Doering, 1874c [1875]: 201.

Bulimus “Macrodonates” cordovanus var. *stelzneri* – Doering, 1875a [1877]: 332; 1875b [1877]: 250.

Odontostomus (Scalarinella) cordovanus var. *stelzneri* – Pilsbry, 1901: 67.

Odontostomus (Scalarinella) cordovanus *stelzneri* – Parodiz, 1939: 732, fig. 2.

Scalarinella (Scalarinella) cordovana stelzneri – Zilch, 1959–1960: 508.

Cyclodontina (Scalarinella) cordovanus stelzneri – Parodiz, 1957b: 29.

Scalarinella (Scalarinella) cordovana stelzneri – Zilch, 1971: 198, pl. 12, fig. 14.

Clessinia stelzneri – Breure, 1974b: 124; – Neubert & Janssen, 2004: 230, pl. 19, fig. 248.

Clessinia cordovana stelzneri – Breure & Schouten, 1985: 9, fig. 3.

Type Locality: “... quebrada de Yatan (Serrezuela; Provincia de Córdoba)”. According to Hylton Scott (1966) the type locality would be located in Argentina, Cordoba Prov., Cruz del Eje Dept., Yatán, Serrezuela.

Type Material: lectotype SMF 10417/3 a; paralectotypes SMF 26582 (1), SMF 26583 (2), SMF 325584 (4).

Distribution: Argentina, *Cordoba Prov.*, Cerro San Vicente (IFML 10739).

Clessinia nattkemperi
(Parodiz, 1944)

Cyclodontina (Scalarinella) nattkemperi Parodiz, 1944: 1–2, figs. A–D; – Parodiz, 1957b: 29; Breure, 1974b: 119.

Clessinia nattkemperi – Fernández, 1973a: 144.

Type Locality: “Pomancillo, 23 km de la ciudad de Catamarca, Republica Argentina”.

Type Material: holotype MACN-In 25713, paratypes MLP 1109.

Distribution: Argentina, *Catamarca Prov.*, Fray Mamerto Esquiú Dept., Pomancillo (IFML 11058, IFML 15186 A, IFML 15185 A, IFML 15764, IFML 15776 A, MLP 11092); Santa María Dept., El Desmonte (IFML 10979).

Clessinia pagoda
Hylton Scott, 1967
Fig. 2A

Clessinia pagoda Hylton Scott, 1967a: 98, figs. 1–6; – Fernández, 1973a: 144; Breure, 1974b: 120; Neubert & Janssen, 2004: 221, pl. 19, fig. 247.

Type Locality: “Quilpo, Sierra Chica de Córdoba”. This locality is part of the Cruz del Eje Dept. in Cordoba Prov., Argentina.

Type Material: holotype MLP 11080; paratypes MACN-In 27284, IFML 14239, SMF 220916 (2), MLP 11076, MLP 11081, MLP 11077, MLP 11078, MLP 11075, MLP 11079.

Distribution: Argentina, *Cordoba Prov.*, Cruz del Eje Dept., Quilpo (MACN-In 27284, IFML 14239, IFML 15178, IFML 1003; IFML 15759 A, MLP 8384, MLP 8387, MLP 8388, MLP 8389, MLP 8386), San Marcos Sierra (IFML 15760 A, IFML 15763, MLP 8390, MLP 8385).



FIG. 2. Type material of Odontostomidae species housed in Argentinean museums. A: *Clessinia pagoda* Hylton Scott, 1967 (paratype IFML 14239); B: *Pilsbrylia hyltonae* Fernández & Rumi, 1980 (lectotype MLP 3991); C: *Spixia cuezzoe* Salas Oroño, 2010 (holotype IFML 15284); D: *Spixia doellojuradoi minor* Parodiz, 1941 (holotype MACN-In 13244); E: *Spixia marmorata* (Hylton Scott, 1971) (holotype MLP 11462); F: *Spixia parodizi* (Hylton Scott, 1952) (holotype MLP 11093); G: *Spixia tucumanensis* (Parodiz, 1941) (holotype MACN-In 23108). Scale bar = 1cm.

Genus *Cyclodontina*
Beck, 1837

Type Species: *Pupa inflata* Wagner in Spix (1827) [subsequent designation by Pilsbry, 1901: 58].

Geographic Distribution: Argentina, Bolivia, Brazil, Paraguay, Uruguay?

Cyclodontina (Bahiensis) guarani
(d'Orbigny, 1835)

Helix guarani d'Orbigny, 1835: 21.

Bulimus guarani – d'Orbigny, 1837 [1834–1847]: pl. 41, fig. 1; 1838: 318; Pfeiffer, 1848: 206; 1853: 427; 1859: 487; 1868: 132.

Pupa (Cyclodontina) guarani – Beck, 1837: 88.

Odontostomus (Bahiensis) guarani – Pilsbry, 1901: 53, pl. 10, figs. 29, 30; Parodiz, 1942b: 332, fig. 15, pl. 3, fig. 25.

Bulimulus guarani – Bertoni, 1926: 73.

Cyclodontina (Bahienesis) guarani – Lange de Morretes, 1954: 70; Parodiz, 1957b: 27; Fernández, 1973a: 120.

Helix guarani – Breure, 1974b: 116.

Cyclodontina (Bahienesis) guarani – Quintana, 1982: 99.

Bahiensis guarani – Breure & Ablett, 2011: 4, fig. 18 A–B iii.

Type Locality: “provincia Corrientesensi (república Argentina)”. Parodiz (1957b) clarified that the type locality of this species is on Parana River at Corrientes- Misiones border, most probably between Ituzaingó and Posadas.

Type Material: lectotype NHMUK 1854.12.4.140.

Distribution: Paraguay; Argentina, (MCZ 182046). *Corrientes Prov.* (MLP 8431), Ituzaingó Dept., Puerto Luján (MACN-In 560); *Santo Tomé Dept.*, Galarza (MLP 8439). *Misiones Prov.*, Apóstoles Dept., Apóstoles (MLP 8437); *Candelaria Dept.*, Profundidad (MACN-In 19886, MACN-In 35901, MLP 8434); *Capital Dept.* Posadas (MLP 8438, MLP 8443); *El Dorado Dept.*, El Dorado (MLP 8442); *Iguazú Dept.* Paraná River near Iguazú

falls (FLMNH 1325649, Pto. Bertoni (MACN-In 9496-2); San Ignacio Dept., San Ignacio (MACN-In 23118, MACN-In 16984); San Javier Dept. (MACN-In 36890); 25 de Mayo Dept., Pto. Londero (MACN-In 6654).

Cyclodontina (Ventania) avellanadae
(Doering, 1881)
[new combination]

Eudioptus avellanadae Doering, 1881: 64, pl. 1, figs. 2, 3.

Anctus (?) stearnsianus – Pilsbry, 1896a: 41.

Odontostomus (Spixia) avellanadae – Pilsbry, 1901: 91, pl. 11, figs. 56–59.

Odontostomus (Ventania) avellanadae – Parodiz, 1940: 229, fig. 5; 1942b: 334, pl. 3, fig. 27.

Cyclodontina (Ventania) avellanadae – Parodiz, 1944: 5; 1957b: 28.

Anctus (?) – stearnsianus – Baker, 1963: 230.

Cyclodontina (Ventania) avellanadae – Fernández, 1973a: 121.

Eudioptus avellanadae – Breure, 1974b: 112.

Type Locality: "... rocas cuarcíticas de la Sierra de Currumalan". Doering (1881) added that later he also found this species in mountains of Sierra de La Ventana.

Type Material: Not located.

Distribution: Argentina, *Buenos Aires Prov.*, Abra de La Ventana (MACN-In 26628), Pileta de los Tres Ríos (IFML 295), Sierra de La Ventana (MACN-In 9487, MACN-In 11270, MACN-In 21163, MACN-In 23090, MACN-In 1644, MACN-In 24395, MACN-In 21263, MACN-In 94874, MACN-In 26628, MCZ 182051, MLP 8433, MLP 8432, MLP 8434, MLP 8435), Sierra de Cura Malal (MACN-In 11085, MACN-In 18196, MACN-In 14201, MLP 1611).

Remarks

The phylogenetic affinity of this monotypic subgenus is currently unknown, showing similar shell morphology to some species classified into *Cyclodontina (Bahiensis)*. However, *Cyclodontina (Ventania) avellanadae* is endemic to an isolated mountain system region in Buenos Aires Province together with *Plagiodontes rocae* and *P. patagonica*. We maintained the classification of this species in a different subgenus because its occurrence is far away from any known species belonging to *Cyclodontina (Bahiensis)*, awaiting a taxonomic revision of this subgenus.

Genus *Odontostomus*
Beck, 1837

Type Species: *Helix gargantua* Férussac, 1821 [synonym: *Bulimus odontostomus* G. B. Sowerby I, 1824].

Geographic Distribution: Argentina, Paraguay, Brazil.

Remarks

The type species, *Odontostomus odontostomus*, was established by tautonymy although the availability of the name is not affected by tautonymy (Art. 18, ICZN).

Holmberg (1909b) described *Odontostomus icareus* from Santiago del Estero Province (syntypes: MACN-In 1365). *Odontostomus icareus* has been cited in previous catalogues (Fernández, 1973a; Breure, 1974b) as a species currently living in Argentina. After examining the type material in MACN, we realize that *O. icareus* has been misidentified and clearly belongs in the genus *Plagiodontes*, probably to *P. daedaleus* distributed in Santiago del Estero Province, also type locality of *O. icareus*.

Odontostomus gargantua
(Férussac, 1821)

Helix (Cochlogena) gargantua Férussac, 1821: 62.

Bulimus odontostoma G. B. Sowerby I, 1824: 59, pl. 5; – Reeve, pl. 38, fig. 228.

Odontostomus gargantula – Beck, 1837: 54 [incorrect subsequent spelling].

Macrodonates sowerbeyii – Swainson, 1840: 334.

Macrodonates odontostomus – Pfeiffer, 1855: 149; Pilsbry 1901: 31, pl. 13, figs. 85–87.

Odontostomus (Macrodonates) Jörgensenianus – Holmberg, 1912b: 153, figs. 11–13.

Odontostomus (Euodontostomus) joergensenianus – Holmberg, 1912b: 153.

Macrodonates thielei – Pilsbry, 1930: 360, pl. 31, fig. 1.

Macrodonates odontostomus jorgensenianus – Parodiz, 1942b: 338, pl. 3, fig. 24.

Odontostomus odontostomus jorgensenianus – Parodiz, 1957b: 29; Fernández, 1973a: 119.

Odontostomus (Euodontostomus) jorgensenianus – Breure, 1974b: 117.

Odontostomus (Odontostomus) gargantua – Quintana, 1982: 98.

Odontostomus gargantua – Breure & Schouten, 1985: 22; Breure & Ablett, 2011: 30, fig. 24 A–D i.

Type Locality: “Rio de Janeiro”.

Type Material: possible syntypes NHMUK 20100632.

Distribution: Paraguay; Brazil; Argentina, *Misiones Prov.*, Cainguás Dept., Dos de Mayo (IFML 11213); Candelaria Dept., Cerro Corá (MLP 9579), Colonia Bonpland (MACN-In 1515); Iguazú Dept., Puerto Aguirre (IFML 073); Guaraní Dept., El Soberbio (IFML 11214); Oberá Dept., Oberá (MACN-In 36895).

Remarks

Odontostomus jorgensenianus (Holmberg, 1912), cited for Argentina by Parodiz (1957b) and Fernández (1973a), is synonymous of *O. gargantua* (Férussac) because of its shell morphology and size, a synonymy previously established by Quintana (1982). Anatomy of this species is unknown.

Genus *Pilsbrylia* Hylton Scott, 1952

Type Species: *Pilsbrylia paradoxa* Hylton Scott [original designation].

Geographic Distribution: Argentina.

Pilsbrylia paradoxa Hylton Scott, 1952

Pilsbrylia paradoxa Hylton Scott, 1952: 6, pl. 1, fig. 2; – Parodiz, 1957b: 29; Weyrauch, 1964: 59; Fernández, 1973a: 145; Breure, 1974b: 120; Richardson, 1993: 53.

Type Locality: “Las Capillas, Jujuy”.

Type Material: holotype MLP 11337, paratypes MLP 11334(1), MLP 11335(2), MLP 11336(5).

Distribution: Argentina, *Salta Prov.*, Güemes Dept., route 9 (IFML 15761, IFML 832); La Caldera Dept., route 9 (IFML 535, IFML 15231, MACN-In 36894, MACN-In 36890) El Yuto (IFML 951). *Jujuy Prov.* (IFML 15678).

Pilsbrylia hyltonae Fernández & Rumi, 1980 Fig. 2B

Pilsbrylia hyltonae Fernández & Rumi, 1980: 75, fig. 1–3.

Pilsbrylia hiltoni – Fernández & Rumi, 1984: 219 [incorrect subsequent spelling].

Type Locality: “Cerro Maldonado, Prov. de Salta, Argentina”. Cerro Maldonado is located in Anta Dept.

Type Material: lectotype MLP 3991, paralectotypes MLP 3990 (2) [present designation]. Lectotype: H = 21.3, DM = 7.2, Hap = 7, Dap = 5.1.

Distribution: Argentina, *Salta Prov.*, Anta Dept., El Rey National Park (IFML 15762, MLP 3990, MLP 3991).

Remarks

Fernandez & Rumi designated a “type” and several paratypes in the original species description. However, there is no clear identification of a single specimen as holotype. These two lots contents several specimens later identified as syntypes by an anonymous reviewer. We confirm that these lots are syntypes based on the information provided in the original publication. To avoid the assumption of holotype (Recommendation 73F of the ICZN), a lectotype (MLP 3991) was selected from the syntypes based on their shell measurements coincident with the original description and type locality. The remaining specimens are paralectotypes (MLP 3990).

Pilsbrylia is here considered as a separate genus awaiting a subgeneric revision of *Cyclodontina* (*Bahiensis*).

Genus *Plagiodontes* Doering, 1875a [1877]

Type Species: *Helix dentata* W. Wood, 1828 [subsequent designation by Pilsbry, 1898a].

Geographic Distribution: Argentina, Uruguay.

Plagiodontes brackebuschii (Doering, 1877)

Bulimus (*Plagiodontes*) *brackebuschii* Doering, 1875a [1877]: 321; – Doering, 1875b [1877]: 240.

Bulimus (Plagiodontes) brackebuschii – Kobelt, 1878: 133.

Bulimus brakebuschii – Kobelt, 1880: 288, pl. 9, figs. 8–10 [incorrect subsequent spelling].

Odontostomus (Plagiodontes) brackebuschii – Pilsbry, 1901: 99, pl. 14, figs. 10–12; Holmberg, 1912a: 26; Parodiz, 1939: 728, fig. E; 1942b: 340.

Cyclodontina (Plagiodontes) brackebuschii – Parodiz, 1957b: 28.

Scalarinella (Plagiodontes) brackebuschii – Zilch, 1971: 198, pl. 11, figs. 6, 7.

Plagiodontes brackebuschii – Fernández, 1973a: 145; Neubert & Janssen, 2004: 202, pl. 20, fig. 252.

Odontostomus brackebuschii – Breure, 1974b: 112; Richardson, 1993: 44.

Type Locality: “... Sierra de S. Luis, cerca de S. Francisco”.

Type Material: lectotype SMF 9350; paralectotype SMF 9351.

Distribution: Argentina, *Cordoba Prov.*, Pocho Dept., Chancani (MLP 12549, MLP 12550, MLP 12548, MLP 12551); Sobremonte Dept., Minas de Cama Cortada (MLP s/n). *Santiago del Estero Prov.*, Sierra de Guasayán (IFML 769, 1610, MLP 12572).

Plagiodontes daedaleus
(Deshayes, 1851)

Pupa daedalea Deshayes in Férussac & Deshayes, 1851: 217, pl. 162, figs. 23, 24.

Bulimus daedaleus – Pfeiffer in Küster & Pfeiffer, 1852: 194 pl. 56, figs. 11–14; Pfeiffer, 1853: 370; 1855: 149; Hidalgo, 1870: 51; Kobelt, 1880: 286, pl. 9, figs. 1–7.

Bulimus (Odontostomus) daedaleus – Doering, 1874b [1875]: 453.

Odontostomus daedaleus – Doering, 1874c [1875]: 198.

Bulimus (Plagiodontes) daedaleus – Doering, 1875b [1877]: 239; Kobelt, 1878: 133; Doering, 1878: 320.

Odontostomus (Plagiodontes) daedaleus – Pilsbry, 1901: 97, pl. 14, figs. 1, 2, 8; – Parodiz, 1939: 721, fig. A, B; 1942b: 340.

Odontostomus (Plagiodontes) daedaleus var. *major* – Doering, 1875b [1877]: 239; 1875a [1877]: 320; Pilsbry, 1901: 99, pl. 14, figs. 6, 7.

Odontostomus (Plagiodontes) daedaleus var. *salinicola* – Doering, 1875b [1877]: 240; Pilsbry, 1901: 98, 99.

Odontostomus (Plagiodontes) daedaleus var. *minor* – Pilsbry, 1901: 99, pl. 14, figs. 4, 5; Parodiz, 1939: 721.

Odontostomus (Plagiodontes) daedaleus var. *multidentatus* – Pilsbry, 1901: 99, pl. 14, fig. 9.

Plagiodontes daedaleus costatus – Hylton Scott, 1952: 16, pl. 2, fig. 1; Fernández, 1973a: 148.

Cyclodontina (Plagiodontes) daedalea – Parodiz, 1957b: 28.

Plagiodontes daedaleus – Fernández, 1973a: 146; Cazzaniga & Fernández Canigia, 1985: 38; Richardson, 1993: 53; Neubert & Janssen, 2004: 217, pl. 20, figs. 250, 251.

Plagiodontes dealdalea – Breure & Schouten, 1985: 16, fig. 9.

Plagiodontes multidentatus – Neubert & Janssen, 2004: 218, pl. 20, fig. 253 [*partim*].

Plagiodontes daedaleus daedaleus – Pizá & Cazzaniga, 2010: 4, figs. 4, 6–9, 15, 18, 19, 22, 24, 28, 29, 36, 37.

Type Locality: “Brazil”. This type locality given by Deshayes is erroneous, as previously stated by Pilsbry (1901), Parodiz (1939) and Pizá & Cazzaniga (2010). *P. daedaleus* is a common species in central and northwestern Argentina.

Type Material: Not located.

P. daedaleus minor: lectotype SMF 9352; *P. daedaleus major*: lectotype SMF 9353; *P. daedaleus multidentatus*: lectotype SMF 9483; *P. daedaleus costatus*: paratype IFML 733/1.

Distribution: Argentina, *Catamarca Prov.* (IFML 15746), El Totoral (IFML 777, IFML 15750), Ambato Dept., Rodeo (IFML 431, IFML 431), El Alto Dept., Guayampa (IFML 448), El Alto (IFML 450); Belén Dept., El Durazno (IFML 7778), Huaycama (IFML 15744). *Cordoba Prov.*, Calamuchita Dept., Embalse (IFML 1277); Cosquin Dept., Mina don Rodolfo (IFML 999); Cruz del Eje Dept., Villa de Soto (MLP 6693); El Totoral Dept., Villa El Totoral (IFML 14754); Ischilín Dept., Copacabana (MLP s/n), Rio Seco (MACN-In 29381), Los Pozos (MACN-In 13240), Punilla Dept., Carlos Paz (MACN-In 26589), La Falda (MACN-In 23555, MACN-In 1874), Pampa de Olaen (MLP 9736), Rio Cuarto Dept., Corte Barrancas (IFML 109); San Javier Dept., Yacanto (IFML 14780), Santa María Dept., Alta Gracia (MACN-In 14204, MACN-In 11755, MACN-In 14212, MACN-In 25950), Totoral Dept.,

Macha (IFML24, IFML 15742). *Salta Prov.*, Cafayate Dept., Santa Bárbara (IFML 42); La Candelaria Dept. (IFML 15756), Unquillo (IFML 124); Anta Dept., del Valle River (IFML 221). *Santiago del Estero Prov.*, Pellegrini Dept., Cerro del Remate (IFML 43). *Tucuman Prov.*, Burruyacú Dept., Alto de Medina (IFML 528), Río Nio (IFML 6135, IFML 15748); Trancas Dept., six kilometers from San Pedro de Colalao (IFML 868), on the way to El Jardín (IFML 11308), Agua Rosada (IFML 430), three kilometers from San Pedro de Colalao (IFML 15754), El Nogalito (IFML 821, IFML 15752), San Pedro de Colalao (IFML 14207).

Plagiodontes dentatus
(W. Wood, 1828)

- Helix sowerbyana* Férussac, 1821: 2; – d'Orbigny, 1835: 22 (*nomen nudum*).
Helix dentata W. Wood, 1828: 50, pl. 8, fig. 71; – Coan & Petit, 2011: 51.
Cyclodontina sowerbyana – Beck, 1837: 88.
Cyclodontina brasilian – Beck, 1837: 88.
Pupa sowerbyana – d'Orbigny, 1837 [1834–1847]: pl. 41, fig. 15; 1838: 321.
Pupa labyrinthus Anton, 1839: 47 (*nomen nudum*).
Bulimus dentatus – Pfeiffer, 1848: 86, 87; 1853: 369; 1855: 149; 1859: 438; – Hidalgo, 1870: 80; Pfeiffer, 1877: 107; Reeve, 1849: 38, fig. 233.
Pupa dentata – Deshayes in Férussac & Deshayes, 1851: 218, pl. 218, fig. 20, pl. 162, figs. 17, 18; Breure, 1974b: 115.
Odontostomus dentatus – Martens, 1868: 207; Strobel, 1874: 17.
Bulimus (Odontostomus) dentatus – Doering, 1874c [1875]: 197; 1874b [1875]: 452.
Bulimus (Plagiodontes) dentatus – Doering, 1875a [1877]: 319; Kobelt, 1878: 133.
Plagiodontes dentatus – Doering, 1881: 69; Breure, 1974b: 110; Fernández, 1973a: 148; Pizá & Cazzaniga, 2003: 6, figs. 6, 8, 14; 2009: 1452, figs. 17, 18–20.
Odontostomus (Plagiodontes) dentatus – Pilsbry, 1901: 94, pl. 14, figs. 15–17, pl. 15, fig. 25; Parodiz, 1939: 715, fig. G; 1942b: 340.
Odontostomus (Plagiodontes) teisseirei Marshall, 1931: 718.
Cyclodontina (Plagiodontes) dentata – Parodiz, 1957b: 28.
Plagiodontes dentata – Breure & Ablett, 2011: 15, fig. 25 A–D i.

Type Locality: Montevideo, Uruguay. Locality fixed by Klappenbach & Olazarri (1973).

Type Material: syntypes NHMUK 1840.9.12.50(2).

Distribution: Uruguay; Argentina, *Buenos Aires Prov.*, Bahía Blanca (MLP 2534, MLP 36085), Punta Piedras (MLP 9754, MACN-In 14739, MACN-In 14514, MACN-In 9713). *Entre Ríos Prov.*, Colón Dept., Colón (MLP 9751); Gualeguaychú Dept., Gualeguaychú (MLP 9749, MLP 36087, MLP 9756); Tala Dept., Gobernador Echagüe (MLP 1609, MLP 16670); Uruguay Dept., Concepción del Uruguay (MLP 9752, MLP 9750); Victoria Dept., Victoria (MLP 36084, MLP 2340, MLP 12648).

Plagiodontes multiplicatus
(Doering, 1874b [1875])

- Bulimus (Odontostomus) multiplicatus* Doering, 1874c [1875]: 196; – Doering, 1874b [1875]: 452; Kobelt, 1876: 5.
Bulimus multiplicatus – Pfeiffer, 1877: 612.
Odontostomus (Plagiodontes) multiplicatus – Pilsbry, 1901: 101.
Odontostomus multiplicatus – Holmberg, 1912a: 25.
Odontostomus (Plagiodontes) multiplicatus – Parodiz, 1939: 718, fig. C; 1942b: 340.
Cyclodontina (Plagiodontes) multiplicatus crassus Hylton Scott, 1948a: 236.
Plagiodontes multiplicatus parvus Hylton Scott, 1952; – Fernández, 1973a: 152; Pizá & Cazzaniga, 2009: 1458, figs. 17, 19, 21.
Cyclodontina (Plagiodontes) multiplicata – Parodiz, 1957b: 28.
Cyclodontina (Plagiodontes) multiplicata parva – Parodiz, 1957b: 28.
Cyclodontina (Plagiodontes) multiplicata crassa – Parodiz, 1957b: 28.
Plagiodontes multiplicatus crassus – Fernández, 1973a: 151; Pizá & Cazzaniga, 2009: 1434.
Plagiodontes multiplicatus – Fernández, 1973a: 150; Breure, 1974b: 119; Pizá & Cazzaniga, 2003: 9, figs. 7, 10, 12, 15; 2006: 93; 2009: 1437.

Type Locality: "... Cerro de Chepe, en la provincia de la Rioja". The Cerro de Chepe is located in Rosario Vera Peñaloza Dept., in La Rioja province.

Type Material: Not located.

Distribution: Argentina, *Catamarca Prov.* (MLP 9759, MACN-In 25748, MACN-In 9843, MLP 9772), Andalgalá Dept. (MLP s/n); Concep-

ción Dept., Chumbicha (MACN-In 9429, MACN-In 9229, MLP 12567); Paclín Dept, La Merced (MACN-In 15709), La Viña, (MLP 1285). *Cordoba Prov.* (MLP 36034), Colón Dept., Unquillo (MLP 9769), Cruz del Eje Dept. (MLP 1626, MACN-In 1637); Ischilín Dept., Quilino (MLP 36063, MLP 1585); Punilla Dept., Cosquín (MLP 12559); Río Seco Dept., Cerro Colorado (MLP 36077, MLP 1319); Tulumba Dept., Lucio Mansilla (MLP 1621), San José de la Dormida (MLP 9768). *La Rioja Prov.* (MLP 9758); Quebrada de la Cébila (MLP 9764), Capital Dept. (MLP 1583); General. Belgrano Dept., Alta Oita (MACN-In 18324, MACN-In 24392); General Ocampo Dept., Ambil (MLP 36081, MLP 36071, MLP 1580); Rosario Vera Peñaloza Dept., Chepes (MLP 9760), *Santiago del Estero Prov.*, Sierra de Guasayán (MACN-In 25845), Colonia Victoria (MLP 9765), Banda Dept., Negra Muerta (MLP 36082); Quebrachos Dept., Sumampa (MACN-In 25850); Río Hondo Dept., Río Hondo (MACN-In 6239), Sarmiento Dept., Villa Matará (MLP 38069, MACN-In 18250).

Remarks

Plagiodontes multiplicatus parvus Hylton Scott, 1952, and *Plagiodontes multiplicatus crassus* (Hylton Scott, 1948) are two published subspecies and were considered as valid biological units by Pizá & Cazzaniga. The type locality *Plagiodontes m. crassus* is “India Muerta, Asusques, Santiago del Estero prov.” (paratypes MLP 11346) and the type locality of *P. m. parvus* (holotype MLP 11348; paratype MLP 11343) is “Cerro Colorado, Salta, Argentina”.

Plagiodontes patagonicus
(d'Orbigny, 1835)

Helix patagonica d'Orbigny, 1835: 22; – Breure, 1974b: 120.

Cyclodontina patagonica – Beck, 1837: 88.

Pupa sowerbyana var. *patagonica* – d'Orbigny, 1838 [1834–1847]: 321.

Bulimus patagonicus – Pfeiffer, 1859: 438.

Bulimus dentatus var. *patagonicus* – Strobel, 1874: 18.

Bulimus (Odontostomus) patagonicus – Doering, 1874b [1875]: 453.

Bulimus (Plagiodontes) patagonicus – Doering, 1875a [1877]: 321; 1875b [1877]: 240; Kobelt, 1878: 133.

Plagiodontes patagonicus – Doering, 1881: 68; Breure, 1975: 1159, pl. 5, fig. 4, pl. 10, fig. 4; Cazzaniga, Pizá & Ghezzi, 2005: 2203, figs. 2, 6–9; Pizá & Cazzaniga, 2009: 1461, figs. 18, 22.

Odontostomus (Plagiodontes) iheringi – Pilsbry & Vanatta in Pilsbry, 1898b: 473.

Odontostomus (Plagiodontes) patagonicus – Pilsbry, 1901: 95, pl. 14, figs. 20–24; – Parodiz, 1939: 720, fig. F; 1942b: 340; Richardson, 1993: 56.

Cyclodontina (Plagiodontes) patagonicus magnus Parodiz, 1957b: 28.

Plagiodontes patagonicus magnus – Fernández, 1973a: 152.

Plagiodontes magnus – Cazzaniga & Fernández Carnigia, 1985: 44.

Type Locality: “Patagonia”.

Type Material: lectotype MNHN 21096; paralectotypes MNHN 21097(1), NHMUK 1854.12.4.235 (5).

Distribution: Argentina, *Buenos Aires Prov.*, Argerich (MLP 9774), Bahía Blanca (MACN-In 34945, MLP 6631, MLP 9775), Cural Malal (MLP 1561), Pigüe (MLP 3046), ruta Nacional 33 (MACN-In 34944, MLP 6628), Sauce Chico (MLP 1574), Sierra de la Ventana (MLP 9776, MLP 9773). *Rio Negro Prov.*, El Fuerte (MLP 3054).

Plagiodontes rocae
Doering, 1881

Plagiodontes rocae Doering, 1881: 65, pl. 1, figs. 5, 6; – Pilsbry, 1901: 97, pl. 14, figs. 18, 19.

Odontostomus (Plagiodontes) rocae – Parodiz, 1939: 718; 1942b: 340.

Cyclodontina (Plagiodontes) rocae – Parodiz, 1957b: 28.

Plagiodontes rocae – Fernández, 1973a: 153; Breure, 1974b: 122; Pizá & Cazzaniga, 2006: 93, figs. 6–9, 11; 2009: 1463, figs. 16, 18, 23, 24.

Type Locality: “... falda meridional de la sierra de Currumalan ...”. The Sierra de Cura Malal is located in Buenos Aires Prov.

Type Material: Not located.

Distribution: Argentina, *Buenos Aires Prov.*, Sierra de Cura Malal (MACN-In 14199, MACN-In 11084, MLP 12564); Sierra de la Ventana (MACN-In 9844).

Plagiodontes strobilii
(Doering, 1875a [1877])

Bulimus (*Odontostomus*) *daedaleus* var. *major* Strobil, 1874: 16 (subspecific name; *non B. daedaleus major* Pfeiffer, 1853); – Pilsbry, 1901: 98 (partim).

Bulimus (*Plagiodontes*) *daedaleus* var. *strobilii* Doering, 1875a [1877]: 320 (replacement name for var. *major* Strobil, 1874; subspecific name); – Doering, 1875b [1877]: 239; Kobelt, 1878: 133.

Bulimus (*Plagiodontes*) *daedaleus* var. *salinicola* Doering, 1875b [1877]: 240; – Doering, 1875a [1877]: 321.

Odontostomus (*Plagiodontes*) *daedaleus* var. *salinicola* – Kobelt, 1878: 133; Pilsbry, 1901: 99.

Cyclodontina (*Plagiodontes*) *daedalea major* – Parodiz, 1939: 721 (partim); 1957b: 28 [partim].

Cyclodontina (*Plagiodontes*) *daedalea minor* – Parodiz, 1939: 721 (partim); 1957b: 28 (partim).

Scalarinella (*Plagiodontes*) *daedaleus strobilii* – Zilch, 1971: 199, pl. 11, fig. 12.

Bulimus (*Odontostomus*) *daedaleus major* – Breure, 1974b: 118.

Plagiodontes daedaleus major – Fernández, 1973a: 147 (partim); Richardson, 1993: 54 (partim).

Plagiodontes strobilii – Neubert & Janssen, 2004: 230, pl. 20, fig. 254; Pizá & Cazzaniga, 2010: 17, figs. 10–14, 20, 21, 25, 26, 30, 31, 38, 40.

Type Locality: "... Cerro de Yerba Buena, en la S. De Aconjigasta ...". The Sierra de Aconjigasta is located in Pocho Dept., in Córdoba province.

Type Material: lectotype SMF 25351.

Distribution: Argentina, *Cordoba Prov.*, West slope of Sierra Grande and Sierra de Comechingones, between Salsacate and Tanginga (IFML 15733). *San Luis Prov.*, Central and northern, San Martín (IFML 15740), 20 kilometers from Santa Rosa (IFML 15729), cerros del Rosario (IFML 15736). *Santiago del Estero Prov.*, Sierra de Guasayán (IFML 15738, IFML 15731).

Plagiodontes weyenberghii
(Doering, 1875a [1877])

Bulimus (*Plagiodontes*) *weyenberghii* Doering, 1875a [1877]: 322; – Doering, 1875b [1877]: 241 (*lapsus calami*); Kobelt, 1878: 133.

Bulimus weyenberghii – Kobelt, 1880: 289, pl. 9, figs. 11, 12; Breure 1974b: 126.

Odontostomus (*Plagiodontes*) *weyenberghii* – Pilsbry, 1901: 100, pl. 14, figs. 13, 14; Holmberg, 1912a: 25.

Odontostomus weyenberghii – Parodiz, 1939: 728, fig. D [incorrect subsequent spelling].

Odontostomus (*Plagiodontes*) *weyenberghii* – Parodiz, 1942b: 340.

Cyclodontina (*Plagiodontes*) *weyenberghii* – Parodiz, 1957b: 28.

Plagiodontes weyenberghii – Hylton Scott, 1965: 24.

Scalarinella (*Plagiodontes*) *weyenberghii* – Zilch, 1971: 199, pl. 11, fig. 12.

Plagiodontes weyenberghii – Fernández, 1973a: 154.

Plagiodontes weyenberghii minor – Fernández, 1973a: 154.

Plagiodontes weyenberghii – Breure & Schouten, 1985: 17, figs. 10, 11.

Odontostomus weyenberghii – Richardson, 1993: 52.

Plagiodontes weyenberghii – Neubert & Janssen, 2004: 235, pl. 20, fig. 255; Pizá & Cazzaniga, 2012: 390, figs. 1–3, 6–7, 9–13.

Type Locality: "...Sierra de Aconjigasta...quebradas del "Nieve" y "Mermela"...". The Sierra de Aconjigasta is located in Pocho Dept., in Córdoba province.

Type Material: lectotype SMF 9354; paralectotype SMF 9355.

Distribution: Argentina, *Cordoba Prov.*, Ongamira (MLP 12555), Gral. San Martín Dept., Villa María (MLP 9833), Pocho Dept., Chancani (MLP s/n), Sierra de Pocho (MLP 13375), Punilla Dept., Capilla del Monte (MLP 2380, MLP 9829), Sobremonte Dept., Chuña Huasi (MLP 9835), San Francisco del Chañar (MLP 12569).

Remarks

Doering (1875) probably made a mistake in writing the species name *weyenberghii*, using an "m" instead of an "n". He not only worked at the same place but personally knew Dr. H. Weyenberg to whom the species is dedicated. For this reason, in accord with previous authors, we considered that this name represents a *lapsus calami*, an error made by the author in writing or misspelling the species name.

Plagiodontes weyrauchi
Pizá & Cazzaniga, 2009

Plagiodontes weyrauchi Pizá & Cazzaniga, 2009: 1438, figs. 4–11.

Type Locality: “Cuesta de la Chilca, 22 km to de East of Andalgalá on the Provincial Road 48 (27°38’19”S, 66°10’27”W), 1455 m about sea level, Catamarca province, Argentina”.

Type Material: holotype MACN-In 37465; paratypes MACN-In 37466 (5), MLP 12673 (3), MLP 12674 (2), IFML 15145 (3); IFML 15145A (2).

Distribution: Argentina, *Catamarca Prov.*, Andalgalá Dept., Cuesta de la Chilca (IFML 15751, IML 640), Aguas de las Palomas (IFML 15749). *Tucuman Prov.*, Trancas Dept., Vupos (IFML 645).

Remarks

Weyrauch cited and wrote on several material labels the name *Plagiodontes pusillus*. However, he never published this name, so it remains unavailable. *Plagiodontes multiplicatus elongatus* Weyrauch is also a *nomen nudum* commonly found on labels handwritten by W. Weyrauch, which Pizá & Cazzaniga (2009) pointed out in describing *Plagiodontes weyrauchi*.

Genus *Spixia*
Pilsbry and Vanatta, 1898

Type Species: *Spixia striata* (Wagner in Spix, 1827) (subsequent designation).

Geographic Distribution: Argentina, Bolivia, Brazil, Paraguay, Uruguay.

Remarks

In 1835 d’Orbigny created *Helix spixii* with two varieties, *minor* and *major*, but did not provide any descriptions, so the names were not available in 1835. In 1837 [1834–1847], d’Orbigny relocated *Helix spixii* in *Pupa*, providing a description of *Pupa spixii* var. *major* (almost smooth, bearing four aperture teeth, shell large and occurring in Bolivia and Corrientes, between the limits of Argentina and Paraguay) and *Pupa spixii* var. *minor* (shell bearing five aperture teeth, lower shell length and distributed

in Chiquitos, Bolivia and northwestern Argentina). Unfortunately, d’Orbigny never selected a nominotypical variety (now subspecies), and *minor* and *major* were very different from each other. d’Orbigny (1837, 1838 [1834–1847]) indicated that *Clausilia striata* Wagner in Spix, 1827, was a synonym of *Pupa spixii*. Pilsbry (1901) decided that *Helix spixii* of d’Orbigny (1835) was based solely upon *Clausilia striata*, which is synonymous of *H. spixii* var. *major*, but not of *H. spixii* var. *minor*, which is substantially different. However, when Pilsbry & Vanatta (1898) created the subgenus *Spixia*, they indicated that *Odontostomus (Spixia) spixii* (= *wagneri* Spix) was the type species of the subgenus, but did not mention the varieties. Pilsbry (1901) and later Parodiz (1942) continued with the use of *Odontostomus (Spixia) spixii* as the type species of the formerly subgenus *Spixia*, arguing that *striata* was preoccupied. Later, Breure & Schouten (1985) clarified that the specific name *striata* is not preoccupied, as has already been noted by Parodiz (1942a), and therefore there is no reason to give preference to the name *spixii* over *striata*. Breure & Ablett (2012) designated a lectotype for each subspecies, *S. spixii major* and *S. spixii minor*, and elevated the latter subspecies to species rank in the genus *Cyclodontina*.

Spixia achalana
(Doering, 1875a [1877])

Bulimus (Odontostomus) achalanus Doering, 1875a [1877]: 324; – Doering, 1878: 134.

Bulimus (Odontostomus) popanus Doering, 1875a [1877]: 325; – Doering, 1878: 134.

Odontostomus (Spixia) achalanus – Pilsbry, 1901: 78; Parodiz, 1942b: 326, fig. 13.

Odontostomus (Spixia) popanus – Pilsbry, 1901: 77; Parodiz, 1942b: 328.

Cyclodontina (Spixia) achalana – Parodiz, 1957b: 27.

Cyclodontina (Spixia) popana – Parodiz, 1957b: 27.

Scalarinella (Spixia) achalana – Zilch, 1971: 199, pl. 12, fig. 16.

Scalarinella (Spixia) popana – Zilch, 1971: 202, pl. 12, fig. 18.

Spixia achalana – Fernández, 1973a: 122.

Spixia popana – Fernández, 1973a: 134.

Odontostomus achalanus – Breure, 1974b: 111.

Odontostomus popanus – Breure, 1974b: 121.

Spixia achalana – Neubert & Janssen, 2004: 197, pl. 18, fig. 219.

Type Locality: "Sierra de Achala (Quebrada de Musi)". This locality is located in Argentina, Cordoba Prov.

Type Material: lectotype SMF 9333a; paralectotypes SMF 9333b (4), MLP 11457 (2).

Bulimus (Odontostomus) popanus: lectotype SMF 9349.

Distribution: Argentina, *Cordoba Prov.*, Cerro San Vicente (IFML 15741), Pocho Dept., Sierra de Pocho (SMF 9349/1, IFML 15370 A), Quebrada de Musi (ZSM s/n); Punilla Dept., Capilla del Monte (MLP 10251); Totoral Dept., Los Mistoles (MACN-In 14842); Ischilín Dept., Deán Funes (MACN-In 9226/31); San Alberto Dept., Sierra de Achala (SMF 9333 a, SMF 9333b ZMB 28505, ZSM s/n, MLP 11457); Santa María Dept., Malagueño (FMNH 107158).

Spixia aconjigastana
(Doering, 1875a [1877])

Bulimus (Odontostomus) aconjigastanus Doering, 1875a [1877]: 326; – Doering, 1875b [1877]: 245; 1878: 134.

Odontostomus (Spixia) aconjigastanus – Pilsbry, 1901: 76; Parodiz, 1942b: 209, pl. 4, fig. 34.

Cyclodontina (Spixia) aconjigastana – Parodiz, 1957b: 27.

Spixia aconjigastana – Hylton Scott, 1965: 24; Fernández, 1973a: 122; Breure & Schouten, 1985: 11, fig. 4.

Scalarinella (Spixia) aconjigastana – Zilch, 1971: 200.

Odontostomus aconjigastanus – Breure, 1974b: 111.

Spixia aconjigastanus – Neubert & Janssen, 2004, 197, pl. 18, fig. 225.

Type Locality: Not mentioned by either Doering (1875a [1877]) or Doering (1875b [1877]). Pilsbry (1901) stated that the type locality of this species is *Sierra de Aconjigasta*.

Type Material: lectotype SMF 9339a; paralectotypes SMF 9339 (7), SMF 29321 (20).

Distribution: Argentina, *Cordoba Prov.* (ZMB s/n), La Toma (IFML 15727), Colón Dept., Unquillo (ZSM 10404, FLMNH 109753, ANSP 226045, MLP 1488); Ischilín Dept., Deán Funes (FMNH 50783); Jesus María Dept., Jesus María (MACN-In 9373); Pocho Dept., Sierra de Mojigasta (SMF 9339 a, b/5, SMF 9339c/3, FMNH 31430), Sierra de Pocho (IFML 15739),

Chancani (MLP 10289, MLP 10254); Punilla Dept., Capilla del Monte (IFML 15215 A, IFML 15725).

Spixia albostrata
Fernández, 1971

Spixia albostrata Fernández, 1971: 58, pl. 1 figs. 4–8, pl. 2 figs. 3, 4; – Fernández, 1973a: 123.

Type Locality: "Casas Viejas, Cordoba".

Type Material: holotype MLP 35104.

Distribution: Argentina, *Cordoba Prov.*, Colón Dept., Cabana (IFML 15737); Punilla Dept., Cosquín (MACN-In 1649), from Capilla del Monte to San Marcos Sierras (IFML 15300 A).

Spixia alvarezii
(d'Orbigny, 1835)

Helix alvarezii d'Orbigny, 1835: 22; – Breure, 1974b: 111.

Bulimus alvarezii – d'Orbigny, 1838 [1834–1847]: 319; Pfeiffer, 1848: 139.

Bulimus (Odontostomus) alvarezii – Doering, 1874b [1875]: 454; 1874c [1875]: 193.

Bulimus (Odontostomus) leptodon Martens, 1875: 276; – Pfeiffer, 1876: 107.

Odontostomus (Spixia) alvarezii – Pilsbry, 1901: 84; Parodiz, 1942a: 202, pl. 2, fig. 10.

Odontostomus (Spixia) leptodon – Pilsbry, 1901: 74; Parodiz, 1942a: 196.

Cyclodontina (Spixia) alvarezii – Parodiz, 1957b: 25.

Spixia alvarezii – Fernández, 1973a: 123 [incorrect subsequent spelling].

Spixia alvarezii – Richardson, 1993: 57.

Type Locality: "provincia Entre-Riosensi (Republica Argentina)". In 1837 (1834–1847) d'Orbigny added "Nous l'avons rencontré sur les coteaux qui bordent le Rio Parana, près du village de *Feliciano*, province d'Entre-rios (république Argentine)".

Type Material: Not located.

Distribution: Argentina, *Cordoba Prov.*, Colón Dept., Cabana (MCZ 182047), La Calera (MACN-In 478, MACN-In 23122, MACN-In 9226-2, MLP 6696/3, MCZ 235254, MCZ 263563, MCZ 116989), Río Ceballos (MACN-In 1642, MACN-In 2444), Unquillo (ZSM 10405, MACN-In 30168, MACN-In 9226-30); Ischilín Dept., Sarmiento (MLP 10295);

Río Primero Dept., Cuenca de Río Primero (MACN-In 9950); Punilla Dept., Capilla del Monte (FMNH 104431), Cosquín (MLP 10267, MACN-In 17418); Totoral Dept., Sierra de Macha (IFML 15301 A, IFML 15302 A, IFML 15303 A); Tulumba Dept., Cerro Colorado (MLP 2328), from Deán Funes to Tulumba (IFML 15735). *Santiago del Estero Prov.*, Banda Dept., Negra Muerta (MLP 35070); Ojo de Agua Dept., Sumampa (FLMNH 109754).

Spixia bergii
(Doering, 1875a [1877])

Bulimus (Odontostomus) bergii Doering, 1875a [1877]: 327; – Doering, 1875b [1877]: 246, pl. 4; 1878: 134.

Odontostomus (Spixia) bergii – Pilsbry, 1901: 87.

Odontostomus (Spixia) berghi – Parodiz, 1942b: 329, pl. 3, fig. 26 [incorrect subsequent spelling].

Scalarinella (Spixia) bergii – Zilch, 1971: 200, pl. 12, fig. 23.

Spixia berghi – Fernández, 1973a: 124 [incorrect subsequent spelling].

Odontostomus bergii – Breure, 1974b: 112.

Spixia bergii – Richardson, 1993: 57.

Spixia bergii – Neubert & Janssen, 2004: 201, pl. 18, fig. 221.

Type Locality: Doering gave the type locality for each of the four varieties described in this species: “a. Alta Gracia”, “b. Cuesta de S. Antonio (Sierra Chica)”, “c. Cerro Salado (S. de Aconjigasta, pendiente oeste)” and “c. Pozo de Piedra (S. de Aconjigasta, pendiente Oeste)”. All these localities are in Córdoba Prov.

Type Material: lectotype SMF 9346a; paralectotypes SMF 9346 b(5).

Distribution: Argentina, *Córdoba Prov.* (FMNH 126891, FMNH 119095, FLMNH 178678), Calamuchita Dept., Calamuchita (MACN-In 23592), Embalse Río tercero (MLP 1635, MLP 1636); Colón Dept., La Granja (MACN-In 14203, MLP 1597); Cruz del Eje Dept., Quilpo (MZSP 66705); Pocho Dept., Sierra de Mojigasta (ZMB s/n); Punilla Dept., Carlos Paz (IFML 15197 A, IFML 15198 A, MACN-In 26588, MACN-In 9021); Santa María Dept., Alta Gracia (SMF 9346 a, SMF 9346 b/5, MACN-In 14213); Tercero Arriba Dept., (MACN-In 6572). *San Luis Prov.*, Ayacucho Dept., Cortaderas (MACN-In 23549); Junín

Dept., La Quebrada (MACN-In 1635). *La Rioja Prov.*, Sanagasta, Dept., Quebrada de los Sauces (MACN-In 15707).

Spixia cala
(Hylton Scott, 1952)

Cyclodontina (Spixia) cala Hylton Scott, 1952: 12, pl. 2, fig. 2; – Fernández, 1973a: 125.

Cyclodontina (Spixia) cala – Parodiz, 1957b: 25.

Cyclodontina (Spixia) cala – Breure, 1974b: 113.

Spixia cala – Richardson, 1993: 57.

Type Locality: “Copacabana, Córdoba”. Copacabana is located in Ischilín Dept.

Type Material: holotype MLP 11454.

Distribution: Argentina, *Córdoba Prov.*, Ischilín Dept., Copacabana (MLP 11454).

Remarks

This species is only represented by the holotype, a single dry shell.

Spixia champaquiana
(Doering, 1875a [1877])
[new combination]

Bulimus (Odontostomus) champaquianus Doering, 1875a [1877]: 330; – Doering, 1875b [1877]: 249; 1878: 134.

Odontostomus (Spixia) champaquianus – Pilsbry, 1901: 80.

Odontostomus (Spixia) tumulorum champaquianus – Parodiz, 1942a: 196, pl. 2, figs. 11, 14.

Cyclodontina (Spixia) chancanina – Parodiz, 1948: 1, figs. 1–3.

Spixia champaquiana – Hylton Scott, 1971: 76; Fernández, 1973a: 125; Neubert & Janssen, 2004: 203, pl. 18, fig. 231.

Scalarinella (Spixia) champaquiana – Zilch, 1971: 200, pl. 12, fig. 25.

Odontostomus champaquianus – Breure, 1974b: 113.

Type Locality: Doering did not provide a specific type locality but described all the localities where he collected *S. champaquiana*: “Esta especie se halla muy diseminada en la pendiente Sudoeste de la Sierra de Achala ... en la pendiente Este de la Sierra de Aconjigasta cerca de Nono ... en la Quebrada del Río

Mina Clavero, y se extiende hasta el extreme meridional de la Sierra de Achala, donde la recojí también en la Quebrada de Oyada en la Provincia de San Luis”.

Type Material: lectotype SMF 9332a; paralectotypes SMF 9332b (10).

Distribution: Argentina, *Catamarca Prov.*, Capayán Dept., Chumbicha (MACN-In 9229-10), Concepción (MACN-In 17785). *Cordoba Prov.*, Calamuchita Dept. (IFML 15210); Colón Dept., Río Ceballos (IFML 6); Cruz del Eje Dept., From Cruz del Eje to Deán Funes (IFML 15153 A); Minas Dept., Sierra de Guasapampa (MZSP 66187); Punilla Dept., Cuesta Blanca (IFML 15209, IFML 15150 A, MZSP 66713), from Carlos Paz to La Calera (IFML 15151 A, IFML 15152 A), from cruz del Eje to Charbonier (IFML 10892); San Alberto Dept., Sierra de Achala (SMF 9332 a, SMF 9332b, ZSM s/n), Villa Brochero (MLP 10281, MACN-In 1324), Mina Clavero (FMNH 217119, IFML 13519, IFML 15721); San Javier Dept., La Paz (MACN-In 1219, MACN-In 3212, MACN-In 2464, MACN-In 11751, MACN-In 1652). *La Rioja Prov.* (MACN-In 22325), Famatina Dept. (IFML 15232); General Belgrano Dept., Iliar (MLP 10286, FLMNH 109760), Olta (MLP 10474, MCZ 182050); Sanagasta Dept., Quebrada de Los Sauces (MLP 10280), Villa Sanagasta (IFML 15365). *San Luis Prov.*, Junín Dept., Merlo (MLP 10282, MLP 10285).

Spixia chancanina
(Doering, 1875a [1877])

Bulimus (Odontostomus) chancaninus Doering, 1875a [1877]: 329; – Doering, 1875b [1877]: 248; 1878: 134.

Bulimus (Odontostomus) salinicola Doering, 1875a [1877]: 328; – Doering, 1875b [1877]: 247, pl. 4; 1878: 134.

Bulimus chancaninus – Kobelt, 1880: 290, pl. 9, figs. 13, 14.

Odontostomus (Spixia) chancaninus – Pilsbry, 1901: 86, pl. 12, figs. 78, 79.

Odontostomus (Spixia) salinicola – Pilsbry, 1901: 87; Parodiz, 1942b: 330, pl. 3, fig. 23.

Cyclodontina (Spixia) chancanina – Parodiz, 1948:1; 1957b: 27.

Spixia chancanina – Hylton Scott, 1965: 23; Fernández, 1973a: 126.

Scalarinella (Spixia) chancanina – Zilch, 1971: 200, pl. 12, fig. 17.

Scalarinella (Spixia) salinicola – Zilch, 1971: 203, pl. 12, fig. 31.

Spixia salinicola – Fernández, 1973a: 138.

Odontostomus chancaninus – Breure, 1974b: 113.

Odontostomus salinicola – Breure, 1974b: 123.

Spixia chancanina – Neubert & Janssen, 2004: 204, pl. 18, fig. 227.

Type Locality: “... pendiente Oeste de la S. de Aconjigasta.” Parodiz 1957b interpreted that the Sierra de Aconjigasta is Sierra de Pocho in Cordoba, Argentina.

Type Material: lectotype SMF 9328; paralectotypes SMF 207997 (2).

Distribution: Argentina, *Catamarca Prov.* (MACN-In 921), Paclín Dept., La Merced (IFML 15305); Valle Viejo Dept. (IFML 15367 A), El Portezuelo (IFML 15305 A, IFML 15306 A). *Cordoba Prov.* (FLMNH 178679, FLMNH 178684); Colón Dept., Jesus María (MACN-In 23123); Cruz del Eje Dept., between Cruz del Eje and Capilla del Monte (IFML 15368 A), from Cruz del Eje to Deán Funes (IFML 15304 A), San Marcos Sierras (IFML 15360 A, IFML 15372 A), Soto (MACN-In 1658); Pocho Dept., between Las Palmas and Chancani (IFML 10879, IFML 15699), Chancani (MLP 10289), Sierra de Mojigasta (MACN-In 25867, SMF 9329 a, SMF 9329 b, ZMB 28506), Sierra de Pocho (MNCN 1505/42144). *San Luis Prov.*, Ayacucho Dept., Sierra de Quines (MACN-In 15125). *Santiago del Estero Prov.*, Choya Dept., Cerro Ichagón (MACN-In 13970); Ojo de Agua Dept., Ojo de Agua (IFML 15376 A).

Spixia charpentieri
(Grateloup in Pfeiffer, 1850)
[new combination]

Bulimus charpentieri Grateloup in Pfeiffer, 1850: 14; – Pfeiffer, 1851: 14; 1853: 369; 1855: 149; 1859: 436; 1868: 76; Hidalgo, 1870: 52.

Bulimus (Odontostomus) charpentieri – Doering, 1874c [1875]: 188; 1874b [1875]: 455; 1878: 134.

Odontostomus (Spixia) charpentieri – Pilsbry, 1901: 76, pl. 12, figs. 75–77; Parodiz, 1942a: 204, pl. 4, fig. 28.

Cyclodontina (Spixia) charpentieri – Parodiz, 1946a: 7; 1957b: 25.

Spixia charpentieri – Fernández, 1973a: 127.

Bulimus charpentieri – Breure, 1974 b: 113.
Cyclodontina charpentieri – Breure & Ablett,
 2011: 11, fig. 21 G–I ii.

Type Locality: “Cordoba”. Doering later said that the type locality of this species is “western slopes of the Sierra de Cordoba”.

Type Material: syntype NHMUK 20110386.

Distribution: Argentina, *Catamarca Prov.* (MACN-In 25749), Ambato Dept., La Puerta (IFML 15307 A, IFML 15308 A, MACN-In 30751); Andalgala Dept., Choya (MACN-In 25749). *Cordoba Prov.* (NHMUK 1829, MLP 10296, ZMB s/n, ZSM s/n, MACN-In 23143, MACN-In 23143-1, FMNH 17251, FLMNH 178680), Colón Dept., Cabana (MLP 10297), La Calera (FMNH 107156); Punilla Dept., Capilla del Monte (MACN-In 1505/42144, FLMNH 109758); Carlos Paz (IFML 15732, MLP 10294); Santa María Dept., Alta Gracia (MACN-In 25951, FLMNH 109759), from Anisacate to Los Molinos Dam (IFML 554, IFML 15775), La Paisanita (IFML 13513), Pampa de Bosque Alegre (MACN-In 1650); Tulumba Dept., Deán Funes (MZSP 66188), San José de la Dormida (MLP 35056). *San Luis Prov.*, Ayacucho Dept., Luján (MACN-In 23145).

Spixia columellaris
 (Parodiz, 1941)

Odontostomus (Spixia) columellaris Parodiz, 1941: 94, pl. 7, figs. 1, 3, 4, 6, 7; – Parodiz, 1942a: 218, pl. 1, fig. 4.

Cyclodontina (Spixia) columellaris – Parodiz, 1957b: 26.

Spixia columellaris – Fernández, 1973a: 125.

Odontostomus (Spixia) columellaris – Breure, 1974b: 114.

Spixia columellaris – Richardson, 1993: 57.

Type Locality: “Minas de Cobre, province of Cordoba, Argentina”. Minas de Cobre is located in Calamuchita Dept.

Type Material: holotype MACN-In 23107, paratype MACN-In 23107.

Distribution: Argentina, *Cordoba Prov.*, Calamuchita Dept., Minas de Cobre (MACN-In 23107, MACN-In 23107); Cruz del Eje Dept., Quilpo (MACN-In 27292, MLP 10302); Ischilín Dept., Copacabana (MLP 10304, MLP 10303).

Spixia costellifer
 (Haas, 1936)

Odontostomus (Spixia) costellifer Haas, 1936: 152.

Spixia estherae – Fernández, 1971: 54, pl. 1 figs. 1–3, pl. 2 figs. 2, 3 [new synonymy].

Odontostomus costellifer – Breure, 1974b: 114.

Spixia costellifer – Richardson, 1993: 57; Neubert & Janssen, 2004: 206, pl. 19, fig. 245.

Type Locality: “Mina, Hierrazuela, Argentinien”. The type locality is located in Cruz del Eje Dept., Cordoba province.

Type Material: holotype SMF 10072; paratype SMF 10073 (1).

Spixia estherae: holotype and paratypes MLP 35088.

Distribution: Argentina, *Cordoba Prov.*, Cruz del Eje Dept., Cruz del Eje (IFML 28, MLP 1613), from Charbonier to Copacabana (IFML 15730, IFML 11219), from Cruz del Eje to Charbonier (IFML 15726, MLP 10330), from Cruz del Eje to San Marcos Sierras (IFML 15334 A), from San Marcos Sierras to El Sauce (IFML 15728), La Cumbre (MLP 10329), Minas de Hierazuela (SMF 10072, SMF 10075).

Remarks

Spixia estherae is here considered synonymous of *S. costellifer*. Both were described from Cruz del Eje, the same political department of Cordoba Province. They both show the same number of shell whorls, similar shell high and fusiform shape. The teleoconch sculptured in both species with marked axial costules.

Spixia cuezzoe
 Salas Oroño, 2010
 Fig. 2C

Spixia cuezzae Salas Oroño, 2010: 2 [inadvertent error].

Type Locality: “Argentina, Cordoba Prov., Punilla Dept., road to Capilla del Monte to San Marcos Sierra, 30°48’39”S, 64°37’14”W, 944 m”.

Type Material: holotype IFML 15284; paratypes IFML 15285 (7); Cordoba Prov., Capilla del Monte (MLP 1489, MLP 6685), San Marcos Sierra (MLP 10290).

Distribution: Argentina, *Cordoba Prov.*, Cruz del Eje Dept., Cerro de La Cruz (IFML 15351 A), from Capilla del Monte to San Marcos Sierras (IFML 15345 A), San Marcos Sierras (IFML 15289 A, IFML 15332), Villa del Soto (IFML 15375); Minas Dept., from San Carlos to Villa del Soto (IFML 15357 A); Punilla Dept., road from Capilla del Monte to San Marcos Sierras (IFML 15286 A, IFML 15287 A), 1 km to the north of Capilla del Monte (IFML 15288 A). *Salta Prov.*, Metán Dept., Cabra Corral dam (IFML 15169, IFML 15170).

Remarks

The original name of this species is explicitly dedicated to G. Cuezco (Salas Oroño, 2010), but the spelling has, on the basis of the original publication itself an inadvertent error. The correct name for the present species is here corrected and should be *Spixia cuezzoe* instead of *S. cuezzae*. This change represents the unique exception for Art. 32.5 (Dubois, 2010; Dubois et al., 2011).

Spixia doellojuradoi
(Parodiz, 1941)
Fig. 2D

Odontostomus (Spixia) doellojuradoi Parodiz, 1941: 93, pl. 7, figs. 11, 14, 15, 18; – Parodiz, 1942b: 320, pl. 1, fig. 5.

Odontostomus (Spixia) doellojuradoi minor Parodiz, 1941: 94; – Parodiz, 1942b: 322.

Cyclodontina (Spixia) doellojuradoi – Parodiz, 1957b: 26; 1962b: 69.

Spixia doellojuradoi – Fernández, 1973a: 128; – Breure & Schouten, 1985: 12.

Spixia doellojuradoi minor – Fernández, 1973a: 128.

Odontostomus (Spixia) doellojuradoi – Breure, 1974b: 115.

Odontostomus (Spixia) doellojuradoi minor – Breure, 1974b: 119.

Spixia doellojuradoi – Breure & Schouten, 1985: 12, fig. 5, 6; Richardson, 1993: 58; – Salas Oroño, 2007: 4, figs. 4–14.

Spixia doellojuradoi minor – Richardson, 1993: 58.

Type Locality: “Estación Casa Grande (between Cosquín and La Falda), *Cordoba*”.

Type Material: holotype MACN-In 23124; paratype MACN-In 23124 (1).

Distribution: Argentina, *Cordoba Prov.* (MCZ 132860), Calamuchita Dept., El Sauce (IFML 10509); Colón Dept., La Calera (MCZ 235255); Cruz del Eje Dept., Los Sauces (MLP 2341), Quilpo (IFML 15349, MLP 10313, IFML 1210); Ischilín Dept., Copacabana (MLP 10308, IFML 15347 A), Ischilín (IFML 14744 A), near Ischilín (IFML 15361 A); Punilla Dept., Cosquín (IFML 15722, MNCN 1505/42271, FMNH 217132), Molinari (MCZ 263566), Pampas de Olaén (IFML 14788 A, IFML 14789 A, MLP 10276), San Marcos Sierras to Quilpo (IFML 15349 A), Valle Hermoso (IFML 15773 A).

Remarks

Parodiz (1941) described the subspecies *Odontostomus (Spixia) doellojuradoi minor* (holotype MACN-In 13244; paratype MACN-In 13244) with the type locality “Pampa de Pocho, Province of *Cordoba*” for the smaller specimens found in this area.

Spixia dubia
(Hylton Scott, 1948)

Cyclodontina (Spixia) dubia Hylton Scott, 1948a: 235; – Parodiz, 1957b: 26.

Spixia dubia – Fernández, 1973a: 129.

Cyclodontina (Spixia) dubia – Breure, 1974b: 115.

Spixia dubia – Richardson, 1993: 58.

Type Locality: “Cerro Colorado (Sudeste de la provincial de *Salta* proximo a su limite con Tucuman y Santiago del Estero)”. Cerro Colorado is located in Metán Dept.

Type Material: holotype MLP 11456; paratypes MLP 11455, MLP 11457.

Distribution: Argentina, *Salta Prov.*, Metán Dept., Cerro Colorado (MLP 11456, MLP 11457, MLP 11455, MLP 10316, MLP 10315, IFML 15315 A, IFML 847).

Spixia holmbergi
(Parodiz, 1941)

Odontostomus (Spixia) holmbergi Parodiz, 1941: 92, pl. 7, fig. 2; – Parodiz, 1942a: 217, pl. 3, fig. 3.

Cyclodontina (Spixia) holmbergi – Parodiz, 1957b: 27.

Spixia holmbergi – Fernández, 1973a: 129.

Odontostomus (Spixia) holmbergi – Breure, 1974b: 116.

Spixia holmbergi – Richardson, 1993: 58.

Type Locality: “Minas de Cobre, Province of Cordoba”.

Type Material: holotype MACN-In 23105; paralectotypes MACN-In 23106.

Distribution: Argentina, *Cordoba Prov.*, Cruz del Eje Dept., La Calera (MLP 6696), San Marcos Sierras (IFML 15260 A, IFML 15262 A, IFML 15199, IFML 15162 A). *Catamarca Prov.*, Ambato Dept., Puerta de Ambato (MACN-In 6242).

Spixia kobeltiana
(Kobelt, 1880)

Bulimus Kobeltianus Kobelt, 1880: 291, pl. 9, figs. 15, 16.

Odontostomus (Spixia) kobeltianus – Pilsbry, 1901: 86, pl. 12, figs. 80, 81; Parodiz, 1942b: 331, fig. 14.

Cyclodontina (Spixia) kobeltiana – Parodiz, 1957b: 27.

Scalarinella (Spixia) kobeltiana – Zilch, 1971: 200, pl. 12, fig. 27.

Spixia kobeltiana – Fernández, 1973a: 130; Neubert & Janssen, 2004: 214, pl. 18, fig. 226.

Bulimus kobeltianus – Breure, 1974b: 117.

Spixia kobeltiana “Doering” – Richardson, 1993: 58.

Type Locality: Kobelt did not assign a specific type locality to this species. Pilsbry (1901) mentioned that the type locality is “Argentine Republic”. However, the locality of the lectotype is Argentina, San Luis Prov., Sierra de San Luis near San Francisco.

Type Material: lectotype SMF 9325.

Distribution: Argentina, *Córdoba Prov.*, Pocho Dept., Chancani (MLP 10289, MLP 10287, MLP 10288), Macha (MLP 10263), Sierra de Pocho (SMF 92447, SMF 26590, SMF 92447), Soto (MLP 10264); Cruz del Eje Dept., canteras de Quilpo (IFML 15724). *San Luis Prov.*, La Capital Dept., San Francisco (SMF 9325).

Remarks

Kobelt (1880) attributed *Bulimus kobeltianus* to Doering, but the correct author is Kobelt, who first described the species.

Spixia marmorata
(Hylton Scott, 1971)
Fig. 2E

Cyclodontina (Spixia) marmorata Hylton Scott, 1971: 75, fig. 2a.

Spixia marmorata – Fernández, 1973a: 131; Breure, 1974b: 118; Richardson, 1993: 59; Neubert & Janssen, 2004: 216, pl. 18, fig. 222.

Type Locality: “Cerro Colorado, Sudeste de Salta”. Cerro Colorado is located in Metán Dept.

Type Material: holotype MLP 11462; paratypes SMF 220914 (2), MLP 11467 (2), MLP 11468(1), MLP 11465(1), MLP 11466(1), MLP 11460 (2), MLP 11461(2), MLP 11463(1).

Distribution: Argentina, *Salta Prov.*, Metán Dept., Cerro Colorado (MLP 10321).

Remarks

This species is only known from the type locality, a mountain isolated in southern Salta.

Spixia martensii
(Doering, 1874b [1875])

Bulimus (Odontostomus) martensii; Doering, 1874b [1875]: 455; – Doering, 1874c [1875]: 181, pl. 3; 1878: 134.

Bulimus (Odontostomus) martensii – Kobelt, 1876: 6; Pfeiffer, 1877: 610.

Odontostomus (Spixia) martensii – Pilsbry, 1901: 88; Parodiz, 1942b: 323.

Cyclodontina (Spixia) martensii – Parodiz, 1957b: 26.

Scalarinella (Spixia) martensii – Zilch, 1971: 201, pl. 12, fig. 19.

Spixia martensi – Fernández, 1973a: 131 [incorrect subsequent spelling].

Odontostomus martensii – Breure, 1974 b: 118.

Spixia martensii – Neubert & Janssen, 2004: 216, pl. 19, fig. 242; Salas Oroño, 2007: 9, figs. 15–26.

Type Locality: “Cerrillos graníticos cerca de Totoral (Prov. De Cordoba)”.

Type Material: lectotype SMF 9454a; paralectotypes SMF 9454 b, MACN-In 23139.

Distribution: Argentina, *Catamarca Prov.*, Ancasti Dept., La Falda (IFML 1491); Tinogasta Dept., Santa Cruz (MACN-In 23140). *Córdoba*

Prov. (IFML 2001, IFML 10931, MLP 10271), between Ascochinga and La Cumbre (IFML 10906), from Charbonier to Copacabana (IFML 11225); Calamuchita Dept., Embalse Rio Tercero (IFML 15356 A, MLP 1634); Colón Dept., Cabana (MLP 10265, FMNH 36595), La Calera (MLP 6696, MACN-In 478-2), Salsipuedes (MACN-In 24407); Cruz del Eje Dept., road to Maza (IFML 15369 A); Punilla Dept., between Los Cocos and Capilla del Monte (IFML 15769 A), from Capilla del Monte to La Cumbre (IFML 14784), from Capilla del Monte to Los Cocos (IFML 14784 A), Capilla del Monte (MACN-In 13246, IFML 14790 A, IFML 14787 A, IFML 708, IFML 1158, IFML 15701, IFML 15211, IFML 2825, IFML 2441, IFML 15771 A, MACN-In 31615, MACN-In 30683, MLP 10252, MACN-In 19914, MACN-In 14844), Carlos Paz (IFML 706, MLP 10328, IFML 15344 A, MACN-In 14836, MACN-In 13248, MACN-In 1879, MACN-In 11021-1, MACN-In 23134, MACN-In 23136, MACN-In 478-2), Casa Grande (IFML 14786 A, IFML 14791 A), Cosquín (MCZ 116986), La Cumbre (MACN-In 9226-1, MACN-In 9226-33), La Falda (IFML 15700), Los Cocos (IFML 2826, IFML 14785 A, MACN-In 30684, MACN-In 6582), Pampa de Olaen (MACN-In 1664), Valle Hermoso (IFML 15200); Ischilín Dept., camino a Ongamira (IFML 14876 A), Copacabana (MLP 10255), Ischilín (IFML 15702, IFML 763, MLP 3062); Rio Primero Dept., La Puerta (MLP 1592); Santa Maria Dept., Alta Gracia (MACN-In 14837, MLP 10272); Totoral Dept., Los Mistoles (MACN-In 14835); Tulumba Dept., between San Jose de la Dormida and Tulumba (IFML 15371), San Jose de la Dormida (IFML 14797 A). *Salta Prov.*, Metán Dept., near Juramento River (IFML 14792, IFML 15172).

Spixia minor
(d'Orbigny, 1837)
[new combination]

Helix spixii var. *minor* d'Orbigny, 1835: 21 (*nomen nudum*).

Pupa striata – Wagner in Spix, 1827: 19 (*partim*).
Pupa spixii var. *minor* – d'Orbigny, 1837 [1834–1847]: pl. 41, fig. 11; 1838: 320.

Odontostomus (Spixia) spixii var. *minor* – Pilsbry, 1901: 71, pl. 12, figs. 64, 65.

Odontostomus bergi – Boettger & Rolle in Rolle, 1908: 160.

Odontostomus (Euodontostomus) saltensis – Holmberg, 1912b: 151, figs. 9, 10.

Odontostomus (Spixia) spixi minor – Parodiz, 1942a: 201, pl. 2, fig. 9.

Cyclodontina (Spixia) spixii minor – Parodiz, 1957b: 25.

Spixia spixii minor – Fernández, 1973a: 140; Richardson, 1993: 60.

Helix spixii minor – Breure, 1974b: 119.

Spixia striata minor – Breure & Schouten, 1985: 14.

Cyclodontina minor – Breure & Ablett, 2012: 27, fig. 21 A–F i.

Type Locality: [Bolivia] “province de Chiquitos, entre Santo-Corazon et San-Juan”.

Type Material: lectotype and paralectotypes NHMUK 1854.12.4.231.

Distribution: Argentina, *Formosa Prov.*, Estero Bellaco (MLP 10467). *Salta Prov.* (MACN-In 1885, MACN-In 10017, SMF 227231, SMF 227232/2), La Viña Dept., Alemania (IFML 15290 A, MACN-In 30752, MACN-In 17603, MACN-In 583-1, MLP 10466), General Güemes Dept., from Salta to Güemes (IFML 15683), La Candelaria Dept. (MLP 11682), Quebrada de Río Ceibal (IFML 15684), El Tala (FMNH 217164), Capital Dept., Cerro San Bernardo (IFML 208, MACN-In 1370, MACN-In 14776, MACN-In 23133, MLP 10468). IFML 14081, El Infiernillo.

Spixia multispirata
(Doering, 1875a [1877])

Bulimus (Odontostomus) multispiratus Doering, 1875a [1877]: 326; – Doering, 1875b [1877]: 245; 1878: 134.

Odontostomus (Spixia) multispiratus – Pilsbry, 1901: 89; Parodiz, 1942a: 210.

Cyclodontina (Spixia) multispirata – Parodiz, 1957b: 27.

Scalarinella (Spixia) multispirata – Zilch, 1971: 201, pl. 12, fig. 24.

Spixia multispirata – Fernández, 1973a: 132; Neubert & Janssen, 2004: 218, pl. 18, fig. 228.

Odontostomus multispiratus – Breure, 1974b: 119.

Type Locality: “Pendiente Oeste de la Sierra de Aconigasta (Quebrada de Yatan, de Nieve, Agua de los Oscuros)”.

Type Material: lectotype SMF 9327a; paralectotypes SMF 9327 b (2).

Distribution: Argentina, *Córdoba Prov.* (MACN-In 23125 b), Las Aguilas (MZSP 66708); Colón Dept., Unquillo (MACN-In 23126); Cruz del Eje Dept., Media Naranja (MACN-In 1660); Pocho Dept., between Las Palmas and El Cadillo (IFML 15316 A, IFML 15318 A, IFML 15202 A, IFML 15212 A), Chancani (IFML 15317 A), Sierra de Pocho (IFML 15333 A, MLP 10253); Tulumba Dept., Intihuasi (MACN-In 23125).

Spixia olainensis
(Doering, 1874c [1875])

Bulimus (Odontostomus) olainensis Doering, 1874c [1875]: 192; – Doering, 1874b [1875]: 454; 1878: 133.

Bulimus olainensis – Pfeiffer, 1877: 610.

Odontostomus (Spixia) olainensis – Pilsbry, 1901: 79.

Odontostomus (Spixia) pucuranus olainensis – Parodiz, 1942a: 196.

Scalarinella (Spixia) olainensis – Zilch, 1971: 201, pl. 12, fig. 26.

Spixia pucarana olainensis – Fernández, 1973a: 135.

Odontostomus olainensis – Breure, 1974b: 120.

Spixia olainensis – Neubert & Janssen, 2004: 220, pl. 18, fig. 232.

Type Locality: “Pampas de Olain (Sierra de Cordova, a 900 m de altura)”.

Type Material: lectotype SMF 9343.

Distribution: Argentina, *Cordoba Prov.*, Pocho Dept., Sierra de Pocho (MZSP 66709); Punnilla Dept. (SMF 9343).

Spixia parodizi
(Hylton Scott, 1952)
Fig. 2F
[new combination]

Cyclodontina (Spixinella) parodizi Hylton Scott, 1952: 13, pl. 1, fig. 3; – Parodiz, 1957b: 27; Fernández, 1973a: 121; Breure, 1974b: 120.

Type Locality: “Casas Viejas. Prov. de Cordoba”.

Type Material: holotype MLP 11093; paratypes MLP 11094 (4); IFML 731 (1).

Distribution: Argentina, *Córdoba Prov.*, Ischilín Dept., Casas Viejas (IFML 731), Orcosuni (IFML 15309, IFML 15310, IFML 15312).

Spixia paucidentata
Hylton Scott, 1971

Spixia paucidentata Hylton Scott, 1971: 73, figs. 1–3; – Fernández, 1973a: 133; Breure, 1974b: 120; Richardson, 1993: 59; Neubert & Janssen, 2004: 221, pl. 18, fig. 233.

Type Locality: “El Rodeo, Dto. de Tulumba, Cordoba”.

Type Material: paratypes MLP 11469, MLP 11470, MLP 11471, MLP 11472, MLP 11473, MLP 11474, SMF 220913 (4).

Distribution: Argentina, *Córdoba Prov.*, Tulumba Dept., El Rodeo (MLP 11469, MLP 11470, MLP 11471, MLP 11472, MLP 11473, MLP 11474, SMF 220913/4 a).

Remarks

Holotype was not found at Museo de Ciencias Naturales, La Plata and is possibly lost.

Spixia pervarians
(Haas, 1936)

Odontostomus (Spixia) pervarians Haas, 1936: 151; – Parodiz, 1942a: 210.

Cyclodontina (Spixia) pervarians – Parodiz, 1957b: 27.

Spixia pervarians – Hylton Scott, 1967: 102, fig. 8; Fernández, 1973a: 133.

Odontostomus (Spixia) pervarians – Breure, 1974b: 121.

Odontostomus (Spixia) pervarians – Richardson, 1993: 59.

Spixia pervarians – Neubert & Janssen, 2004: 222, pl. 18, fig. 220.

Type Locality: “Sierra de Achala bei Candelaria, Prov. Salta, Argentinien”. There is an mistake with the location of the type locality since Sierra de Achala is located into Cordoba Province, not in Salta province.

Type Material: holotype SMF 10074; paratypes SMF 10075 (2), SMF 10076 (5).

Distribution: Argentina, *Cordoba Prov.*, San Alberto Dept., Sierra de Achala (SMF 10074, SMF 10075, SMF 10076), Cruz del Eje Dept., Quilpo (IFML 1212, MLP 10334, MLP 10331, IFML 15747), Candelaria (MLP 10336), San Gregorio (MLP 10335), Pocho Dept., Mogigasta (MLP 10333).

Spixia philippii
(Doering, 1874c [1875])

Bulimus (*Odontostomus*) *Philippii* Doering, 1874c [1875]: 180, non Pfeiffer, 1842; – Doering, 1874b [1875]: 456; 1878: 135.

Bulimus (*Odontostomus*) *philippii* – Kobelt, 1876: 8.

Bulimus philippii – Pfeiffer, 1877: 612.

Bulimus (*Odontostomus*) *doeringi* – Kobelt, 1878: 135 (new name for *philippii* Doering 1874, non Pfeiffer, 1842).

Odontostomus (*Spixia*) *philippii* – Pilsbry, 1901: 75, pl. 12, figs. 73, 74; Parodiz, 1942a: 205, pl. 4, fig. 29.

Cyclodontina (*Spixia*) *philippii* – Parodiz, 1957b: 26.

Scalarinella (*Spixia*) *philippii* – Zilch, 1971: 201, pl. 12, figs. 21–22.

Spixia philippii – Fernández, 1973a: 133; – Neubert & Janssen, 2004: 223, pl. 19, fig. 241.

Odontostomus philippii – Breure, 1974b: 121.

Type Locality: “... cerrillos graníticos en la llanura cerca del Totoral, al Norte de la Provincia de Cordoba”.

Type Material: syntypes SMF 92441a, SMF 92441b (11), SMF 9345a (19), SMF 9345b (4).

Distribution: Argentina, *Córdoba Prov.*, (MLP 10344, FLMNH 178681, FLMNH 166156); Cruz del Eje Dept., (SMF 92441 a, MACN-In 378, MACN-In 14846, MLP 2406), Estancia La Fronda (IFML 949), Villa de Soto (IFML 10891), Villa del Soto to Cosquín (IFML 10891), La Loma (MACN-In 1639), Quilpo (MLP 10343); Totoral Dept., (MLP 10327), Villa General Mitre (IFML 15745), Villa del Totoral (IFML 15319, IFML 15320, IFML 15321, IFML 14741, IFML 14742, IFML 14759, IFML 12770, IFML 15348, FMNH 36597, FMNH 72230); Ischilín Dept., Ischilín (IFML 15346, IFML 15362); Punilla Dept., (MLP 35047) San Gregorio (MLP 10345), Capilla del Monte (FMNH 17249, FMNH 107154, FMNH 217122, FMNH 217067, MCZ 113841, MCZ 108690); Pocho Dept., Chancani (MLP 10289); Calamuchita Dept., Calamuchita (FMNH 50802). *Catamarca Prov.*, Ambato Dept., La Puerta (MACN-In 6240).

Remarks

Kobelt (1878) proposed the name *Bulimus* (*Odontostomus*) *doeringii* to replace *Odon-*

tostomus philippii Doering, 1874c [1875], which he thought was preoccupied. Pilsbry (1901) clarified that the changed was unnecessary.

Spixia profundidens
(Doering, 1874c [1875])

Bulimus (*Odontostomus*) *profundidens* Doering, 1874c [1875]: 184; – Doering, 1874b [1875]: 455; 1878: 134.

Bulimus (*Odontostomus*) *maculosus* Doering, 1874c [1875]: 186; – Doering, 1874a: 455; 1878: 134.

Bulimus (*Odontostomus*) *profundidens* – Kobelt, 1876: 7.

Bulimus (*Odontostomus*) *maculosus* – Kobelt, 1876: 7.

Bulimus profundidens – Pfeiffer, 1877: 611.

Bulimus maculosus – Pfeiffer, 1877: 611.

Odontostomus (*Spixia*) *profundidens* – Pilsbry, 1901: 81.

Odontostomus (*Spixia*) *maculosus* – Pilsbry, 1901: 78; Parodiz, 1942a: 208, pl. 2, figs. 13, 16.

Cyclodontina (*Spixia*) *tumulorum profundidens* – Parodiz, 1942a: 196; 1957b: 26.

Cyclodontina (*Spixia*) *maculosa* – Parodiz, 1957b: 26.

Scalarinella (*Spixia*) *profundidens* – Zilch, 1971: 202.

Spixia maculosa – Fernández, 1973a: 131.

Odontostomus profundidens – Breure, 1974b: 121.

Odontostomus maculosus – Breure, 1974b: 118.

Spixia profundidens – Neubert & Janssen, 204: 225.

Type Locality: “... Sierra de Achala [Cordoba], cerca de San Carlos, a 600 metros de altura”.

Type Material: lectotype SMF 9336.

Distribution: Argentina, *Cordoba Prov.*, (SMF 208007/1, FMNH 119267, FMNH 31485); San Alberto Dept., Sierra de Achala (SMF 9336), Ischilín Dept., Ischilín (MACN-In 10319), Ongamira (IFML 15155, IFML 15156, IFML 15157, IFML 15158, IFML 15159); Totoral Dept. (ZMB 28504), Santa Catalina (IFML 15160, IFML 15161, IFML 15355), Sierra Chica (MACN-In 30753); Punilla Dept., Capilla del Monte (MLP 10342), Cerro Uritorco (MZSP 66711).

Spixia pucurana
(Doering, 1874c [1875])

Bulimus (Odontostomus) pucuranus Doering, 1874c [1875]: 183; – Doering, 1878: 134.

Bulimus (Odontostomus) pucaranus – Doering, 1874b [1875]: 456 [incorrect subsequent spelling]; Kobelt, 1876: 8; 1878: 134.

Bulimus pucaranus – Pfeiffer, 1877: 612.

Odontostomus (Spixia) pucuranus – Pilsbry, 1901: 82; Parodiz, 1942a: 214, pl. 4, figs. 36, 37.

Cyclodontina (Spixia) pucarana – Parodiz, 1957b: 26 [incorrect subsequent spelling].

Scalarinella (Spixia) pucarana – Zilch, 1971: 202, pl. 12, fig. 28 [incorrect subsequent spelling].

Spixia pucurana – Fernández, 1973a: 135; – Neubert & Janssen, 2004: 225, pl. 19, fig. 235.

Odontostomus pucaranus – Breure, 1974b: 121.

Type Locality: “... Valle de Río Primero en la Sierra Chica de Córdoba, pero solamente en las regiones intermedias, graníticas ...”.

Type Material: lectotype SMF 9338a; paralectotypes SMF 9338b (2).

Distribution: Argentina, *Catamarca Prov.*, (IFML 15359). *Cordoba Prov.*, (MACN-In 11763, MACN-In 13247, MACN-In 9226), *Punilla Dept.*, Valle Hermoso (MACN-In 3224), *Río Primero Dept.*, (SMF 9338 a, SMF 9338 b, IFML 385); *Cruz del Eje Dept.*, San Marcos Sierras (IFML 15163). *San Luis Prov.*, Capital Dept., Sierra de San Francisco, (MACN-In 15139); *Ayacucho Dept.*, Sierra de Quines (MACN-In 15124).

Spixia pyrgula
(Hylton Scott, 1952)

Cyclodontina (Spixia) pyrgula Hylton Scott, 1952: 8, pl. 1, fig. 2, pl. 2, fig. 3; – Parodiz, 1957b: 26; Breure, 1974b: 122.

Spixia pyrgula – Fernández, 1973a: 136; Breure & Schouten, 1985: 14, fig. 7; Richardson, 1993: 59; Neubert & Janssen, 2004: 226, pl. 19, fig. 238.

Type Locality: “Orcosuni, camino a Dean Funes. Prov. de Cordoba”.

Type Material: holotype MLP 11475; paratypes MLP 10348, MLP 11476, MLP 11477,

MLP 11478, IFML 730, SMF 71806 (8), SMF 164110 (1).

Distribution: Argentina, *Cordoba Prov.*, Ischilín Dept., Orcosuni (SMF 71806/8, SMF 164110, MLP 11745, MLP 11478/2, IFML 730, IFML 15767, MLP 10348/ 6, MLP 11476/2, MLP 11477/6, IFML 15311, MLP 10349, FMNH 72249), *Deán Funes (FMNH 50780)*; *Cruz del Eje Dept.*, Cerro Cuniputo (MACN-In 32860); *Punilla Dept.*, Charbonier (MACN-In 3225).

Spixia pyriformis
(Pilsbry, 1901)

Bulimus (Odontostomus) Doeringii Kobelt, 1882: 5, pl. 1, fig. 6a (*non Bulimus doeringii* Kobelt, 1878).

Odontostomus (Spixia) pyriformis Pilsbry, 1901: 72, pl. 12, figs. 71, 72 (new name for *Bulimus (Odontostomus) doeringii* Kobelt, 1882).

Odontostomus (Spixia) pyriformis – Parodiz, 1942a: 216, pl. 1, fig. 6.

Cyclodontina (Spixia) pyriformis – Hylton Scott, 1948a: 230; Parodiz, 1957b: 26.

Scalarinella (Spixia) pyriformis – Zilch, 1971: 202, pl. 12, fig. 34.

Spixia pyriformis – Fernández, 1973a: 136.

Odontostomus pyriformis – Breure, 1974b: 122.

Spixia pyriformis – Richardson, 1993: 59; Neubert & Janssen, 2004: 226, pl. 19, fig. 239; Salas Oroño, 2007: 15, figs. 27–39.

Type Locality: “Argentina: Sierra de Cordova”. Parodiz (1957b) clarified that the type locality was erroneous because this species is not found in Cordoba province and only in Tucuman and Salta provinces, Argentina.

Type Material: lectotype SMF 171579; paralectotype SMF 171580 (1).

Distribution: Argentina, *Salta Prov.*, Metán Dept., Cerro Colorado (IFML 15743, IFML 14793, IFML 14794, SMF 171579, SMF 171580/1, FMNH 217165, FMNH 217164, MCZ 166880), *Sierra Colorada (IFML 14793, IFML 14795)*. *La Candelaria (IFML 15765)*.

Spixia reticulata
(Doering, 1875b [1877])

Bulimus (Odontostomus) reticulatus Doering, 1875b [1877]: 250, pl. 4; 1875a [1877]: 331; 1878: 134.

Odontostomus (Spixia) reticulatus – Pilsbry, 1901: 88.

Odontostomus (Spixia) reticulatus – Parodiz, 1942a: 207, pl. 4, fig. 37.

Cyclodontina (Spixia) reticulatus – Parodiz, 1957b: 27.

Scalarinella (Spixia) reticulata – Zilch, 1971: 202, pl. 12, fig. 29.

Spixia reticulata – Fernández, 1973a: 137.

Odontostomus reticulatus – Breure, 1974b: 122.

Spixia reticulata – Neubert & Janssen, 2004: 227, pl. 18, fig. 229.

Type Locality: “Esta especie puebla la pendiente Este de la Sierra de Aconjigasta, los altos de la Tablada, Plumería, etc”.

Type Material: lectotype SMF 9326a; paralectotypes SMF 325582 (4).

Distribution: Argentina, *Catamarca Prov.*, Fray Mamerto Esquiú Dept., Dique Las Pirquitas (IFML 15322), Gracián (MACN-In 24390). *Cordoba Prov.*, (MACN-In 23114), Pocho Dept., Sierra de Pocho (SMF 9326 a, SMF 9326 b, IFML 15313 A, ZMB 28502); Tulumba Dept., Intihuasi (MACN-In 23115).

Spixia riojana
(Doering, 1874b [1875])

Bulimus (Odontostomus) riojanus Doering, 1874b [1875]: 454; 1878: 133; – Kobelt, 1876: 6.

Bulimus (Odontostomus) riochanus – Doering, 1874c [1875]: 190 [incorrect subsequent spelling].

Bulimus riojanus – Pfeiffer, 1877: 610.

Odontostomus (Spixia) riojanus – Pilsbry, 1901: 83; Parodiz, 1942b: 327, pl. 4, fig. 33.

Odontostomus (Eudontostomus) riojanus – Holmberg, 1912a: 24.

Cyclodontina (Spixia) riojana – Parodiz, 1957b: 27.

Scalarinella (Spixia) riojana – Zilch, 1971: 202, pl. 12, fig. 30.

Spixia riojana – Fernández, 1973a: 137; Neubert & Janssen, 2004: 227, pl. 18, fig. 224.

Odontostomus riojanus – Breure, 1974b: 122.

Type Locality: “Sierra de La Rioja”.

Type Material: lectotype SMF 9337a; paralectotypes SMF 325581 (2), MACN-In 9127 (3).

Distribution: Argentina, *Catamarca Prov.*, El Alto Dept., El Alto (IFML 435). *La Rioja Prov.*, (SMF 9338 a, SMF 9337 b, MACN-In 9127-3, FLMNH 178683), Castro Barros Dept., (IFML 15327, IFML 15418, IFML 15326), Sierra de Velazques (IFML 15366), Santa Cruz (IFML 15325), road to Anillaco (IFML 15326), Sanogasta Dept., Villa Sanogasta (IFML 15335), from Bazán to Anillaco (IFML 15203).

Spixia striata
(Wagner in Spix, 1827)

Pupa striata Wagner in Spix, 1827: 19 (*partim*).

Helix spixii var. *major* d’Orbigny, 1835: 21 (*nomen nudum*).

Pupa spixii var. *major* d’Orbigny, 1838 [1834–1847]: 320.

Cyclodontina striata – Beck, 1837: 88.

Pupa spixii – Gray, 1854: 23 (*partim*).

Bulimus (Odontostomus) striatus – Pfeiffer, 1855: 149; Doering, 1874b [1875]: 424; 1878: 134.

Bulimus striatus Spix – Pfeiffer, 1859: 437.

Odontostomus (Spixia) spixii var. *major* – Pilsbry, 1901: 70, pl. 12, fig. 66.

Odontostomus (Spixia) spixii var. *paraguayanus* – Pilsbry, 1901: 71, pl. 12, fig. 67, pl. 15, fig. 34.

Odontostomus (Spixia) spixii var. *Bohlsi* – Pilsbry, 1901: 70.

Odontostomus (Spixia) spixii major – Parodiz, 1942a: 201.

Cyclodontina spixii major – Parodiz, 1957b: 25.

Spixia spixii major – Fernández, 1973a: 139; Quintana, 1982: 100.

Spixia striata – Breure, 1975: 1158 (*partim*); Breure & Schouten, 1985: 14, fig. 8; Richardson, 1993: 59; Breure & Ablett, 2012: 26.

Type Locality: “... pays habité par les Guarayos, au sein des forêts humides des frontières nord de la province de Chiquitos (république de Bolivie), et dans la province de Corrientes (république Argentine), en un bois voisin de la rivière de Santa-Lucia, au lieu dit “Pasto reito”” (d’Orbigny, 1838 [1834–1847]).

Type Material: lectotype NHMUK 1854.12.4.232; paralectotypes NHMUK 1854.12.4.232 (6).

Distribution: Bolivia; Paraguay; Brazil?; Argentina, *Corrientes Prov.*, Manantiales (MACN-In 9505). Misiones Prov.? Puerto María (MACN-In 23132).

Spixia subsexdentata
(Doering, 1874b [1875])

Bulimus (Odontostomus) subsexdentatus Doering, 1874b [1875]: 454.

Bulimus (Odontostomus) pseudosexdentatus – Doering, 1874c [1875]: 194, pl. 3.

Bulimus (Odontostomus) subsexdentatus – Kobelt, 1876: 6.

Bulimus subsexdentatus – Pfeiffer, 1877: 609.

Odontostomus subsexdentatus – Doering, 1878: 133.

Odontostomus (Spixia) subsexdentatus – Pilsbry, 1901: 85.

Scalarinella (Spixia) subsexdentata – Zilch, 1971: 203.

Odontostomus subsexdentatus – Breure, 1974b: 124.

Spixia subsexdentata – Neubert & Janssen, 2004: 231, pl. 19, fig. 240.

Type Locality: “La variedad normal es frecuente en los cerritos calcáreos cerca del valle del Río Primero, en la pendiente Oeste de la Sierra de Córdoba”. “La var. *major* parece predominar en el Norte de la Provincia ... cerca de San Pedro”. The localities indicated by Doering are located in Córdoba Prov, Argentina.

Type Material: lectotype SMF 9330.

Distribution: Argentina, *Córdoba Prov.* (SMF 9330/1, SMF 164196/3), Colón Dept., Cabana (IFML 762), Unquillo (MACN-In 21785); Río Seco Dept., Sierra de Ambargasta (IFML 15328 A); Punilla Dept., Valle Hermoso (IFML 15330 A); Santa María Dept., Guayacaste (MLP 1615), Pampa Alta (MLP 10278), Río Ceballos (MLP 10269), San Pedro (FMNH 36594); Tulumba Dept., from Deán Funes to Tulumba (IFML 15194), from San José de la Dormida to Tulumba (IFML 15329 A, IFML 15236, IFML 15373 A, IFML 15374 A), Tulumba (IFML 15238, IFML 10907, FMNH 217157). *Santiago del Estero Prov.*, Banda Dept., Negra Muerta (MLP 35070); Guasayán Dept. (MACN-In 25846); Ojo de Agua Dept., Ojo de Agua (MLP 10270).

Spixia tridens
Hylton Scott, 1967

Spixia tridens Hylton Scott, 1967: 101, fig. 7; – Fernández, 1973a: 140; Breure, 1974b: 125; Richardson, 1993: 61; Neubert & Janssen, 2004: 232, pl. 19, fig. 236.

Type Locality: “Quilpo. Córdoba”. Quilpo is located in Cruz del Eje Dept.

Type Material: paratypes MLP 11479(2), MLP 11480, SMF 220915 (1).

Distribution: Argentina, *Córdoba Prov.*, Cruz del Eje Dept., Quilpo (MLP 11479(2), SMF 220915, IFML 15354).

Spixia tucumanensis
(Parodiz, 1941)
Fig. 2G

Odontostomus (Spixia) tucumanensis Parodiz, 1941: 92, pl. 7, figs. 10, 12, 13, 16; – Parodiz, 1942b: 319, pl. 1, fig. 1.

Cyclodontina (Spixia) tucumanensis – Parodiz, 1957b: 27.

Spixia tucumanensis – Fernández, 1973a: 141. *Odontostomus (Spixia) tucumanensis* – Breure, 1974b: 125.

Spixia tucumanensis – Richardson, 1993: 61; Salas Oroño, 2007: 20, figs. 39–49.

Type Locality: “Margins of Río Salí, near Tucuman city, Argentina”.

Type Material: holotype MACN-In 23108; paratypes MACN-In 23109.

Distribution: Argentina, *Tucumán Prov.*, (MLP 35036, MACN-In 21478, MACN-In 18321, MCZ 132861), Trancas Dept. (IFML 15379, IFML 15355 A), between Tapia and Tafí Viejo (MLP 1606, MLP 35036), between Tapia and Vipos (IFML 326, IFML 10837, IFML 11220), El Cadillal (IFML 14201, MLP 10472), from Tapia to Raco (IFML 326, IFML 14774 A, IFML 15204, IFML 15353), India Muerta river (IFML 15350 A, IFML 15207, IFML 15682), Salí river (MACN-In 9700), San Pedro de Colalao (IFML 15340, IFML 10894, FMNH 217127), San Javier (MACN-In 23110), Ticucho (IFML 15757, IFML 15208, IFML 15380), Vipos (IFML 14770, IFML 15341 A, IFML 15352, IFML 14769, IFML 15377, MACN-In 17581, MLP 10471, FMNH 217166, MLP 10473, IFML 10838).

Spixia tumulorum
(Doering, 1874c [1875])

Bulimus (Odontostomus) tumulorum Doering, 1874c [1875]: 187, – Doering, 1874b [1875]: 456; 1878: 134; Pilsbry, 1901: 81.

Bulimus (Odontostomus) tumulorum – Kobelt, 1876: 7.

Bulimus tumulorum – Pfeiffer, 1877: 611.

Odontostomus (Spixia) tumulorum – Parodiz, 1942a: 211, pl. 2, figs. 11–16.

Cyclodontina (Spixia) tumulorum – Parodiz, 1957b: 26.

Scalarinella (Spixia) tumulorum – Zilch, 1971: 203, pl. 12, fig 32.

Spixia tumulorum – Fernández, 1973a: 141; – Neubert & Janssen, 2004: 233, pl. 19, fig. 237.

Odontostomus tumulorum – Breure, 1974b: 125.

Type Locality: "... cerritos de la Calera cerca del pueblo de Córdoba". The locality indicated by Doering is located in Argentina, Córdoba Prov.

Type Material: lectotype SMF 9331a; paralectotypes SMF 325585 (2).

Distribution: Argentina, Córdoba Prov. (SMF 9331 a, SMF 9331 b, FMNH 31459), Calamuchita Dept., Minas de Cobre (MACN-In 23129), San Javier (MACN-In 14852); Colón Dept., La Calera (MACN-In 23128); Cruz del Eje Dept., La Higuera (IFML 15233 A), Serruzuela (IFML 15155, FMNH 156679), Sierra de Mojigasta (IFML 15154 A); Punilla Dept., Charbonier (IFML 11219). La Rioja Prov., Belgrano Dept., Iliar (MACN-In 18353, FMNH 125190, FMNH 17250, MLP 1484), Olta (MACN-In 13327, MLP 10477); Chamental Dept., Gobernador Gordillo (MCZ 182045, MLP 10476, MLP 10475); Famatina Dept., between Pitul and Campana (IFML 15358), Cuesta de Aguadita (IFML 15213 A, IFML 15364).

Family Simpulopsidae
Schileyko, 1999

Genus *Rhinus*
Albers, 1860

Type Species: *Bulimus heterotrichus* Moricand [original designation].

Geographic Distribution: Argentina, Brazil, Venezuela.

Rhinus argentinus
(Ancey, 1901)

Bulimulus (Rhinus) argentinus Ancey, 1901: 92; – Miquel, 1991: 103; Breure, 2011: 14.

Rhinus argentinus – Breure, 1978: 230, pl. 11, fig. 12; 1979: 130.

Type Locality: "Guaileguaychu, province d'Entrerios, République Argentine".

Type Material: syntypes RBINS/MT 1867 (3).

Distribution: Argentina, Entre Ríos Prov.

Remarks

The three syntypes are labeled as "types" in Ancey's handwriting. *Rhinus* was classified within Simpulopsidae by Breure (2011). No available material is deposited in the Argentinean collections reviewed.

Genus *Simpulopsis*
Beck, 1837

Type Species: *Helix sulculosa* Férussac, 1821 [subsequent designation by Albers, 1860].

Geographic Distribution: Argentina, Brazil, Paraguay, Peru, Ecuador, Colombia, Venezuela, Suriname, Guiana Francesa.

Simpulopsis (Eudioptus) citrinovitrea
(Moricand, 1836)

Helix (Cochlogena) citrino-vitrea Moricand, 1836: 436, pl. 2, fig. 19; – Breure, 1974a: 53.

Bulimulus (Paracochlea) willineri – Hylton Scott, 1967b: 93, figs. 1–4; Fernández, 1973a: 86.

Pseudoglandina agitata – Breure, 1978: 235.

Simpulopsis (Eudioptus) citrinovitrea – Breure, 1978: 234, figs. 411–415; 1979: 135; Neubert & Janssen, 2004: 205, pl. 17, fig. 208.

Simpulopsis (Eudioptus) willineri – Miquel, 1998: 185.

Type Locality: "Brazil, Bahia, near Salvador".

Type Material: syntypes MNHN, SMF 302256 (2).

Distribution: Brazil; Paraguay; Peru; Ecuador; Colombia; Argentina, Misiones Prov. (IFML 15705), San Ignacio (MLP13533). Salta Prov., Anta Dept., El Rey National Park (IFML15704 A, IFML 15703 A, IFML15706 A).

Remarks

Simpulopsis has been previously classified in Amphibuliminae (Zilch, 1959–1960; Breure, 1973); later Breure (1979) included it in Bulimulinae. Bouchet & Rocroi (2005) classified them in the tribu Simpulopsini Schileyko, 1999, of the

subfamily Bulimulinae. Breure (2011) relocated *Simpulopsis* and *Rhinus* in a separate family of the Orthalicoidea, Simpulopsidae Schileyko, 1999.

Simpulopsis (Eudioptus) eudioptus
(Ihering in Pilsbry, 1897)
[new combination]

Bulimulus (Protoglyptus) eudioptus Ihering in Pilsbry, 1897: 89, pl. 14, figs. 16, 17; – Haas, 1959: 367.

Bulimulus (Scansicochlea) jörgenseni Holmberg, 1912b: 150, figs. 7, 8; – Parodiz, 1957a: 134; Quintana, 1982: 90.

Naesiotus eudioptus – Breure, 1979: 69.

Bostryx eudioptus – Miquel, 1995: 120, pl. 1, figs. 1, 2.

Type Locality: “Sao Paulo, Brazil”.

Type Material: holotype ANSP 71240; paratypes ANSP 71240 (3), MZSP 620 (24), MZSP 7735 (2), MZSP 7746 (9), MZSP 3398 (1), MZSP 7737 (3).

Bulimulus (Scansicochlea) jörgenseni: holotype MACN-In 1377.

Distribution: Brazil; Chile; Argentina, *Jujuy Prov.* (MACN-In 15949), *Calilegua Dept.* (MLP 10167). *Misiones Prov.* (MACN-In 12062), *Candelaria Dept.*, *Colonia Bonpland* (MACN-In 1377); *Capital Dept.*, *Iguazú Dept.* (MLP 10166, MLP 10168, MLP 13534); *Libertador Gral. San Martín Dept.*, *Oberá Dept.* (MLP 10165); *San Ignacio Dept.* (MACN-In 12128, MACN-In 13023, MACN-In 16985, MACN-In 9705).

Remarks

Bulimulus eudioptus was originally described in the genus *Bulimulus* and subsequently changed to *Naesiotus* (Breure, 1979) and *Bostryx* (Miquel, 1995). Current classification of *B. eudioptus* into *Simpulopsis (Eudioptus)* is based on that this species shows morphological characters typical of the subgenus: the presence of spiral lines cutting axial wrinkles in the protoconch, fragile, near transparent shell walls, general shell shape and size, and the absence of penis sheath presenting a tunica-like over the distal portion of the reproductive system. Additionally, the pulmonary roof shows the typical dark spots that characterized most of *Simpulopsis* species, and the adrectal ureter is closed over its entire length.

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PREDATION ON RECENT TURRITELLINE GASTROPODS FROM THE INDIAN SUBCONTINENT AND COMPARISON WITH A REVISED GLOBAL DATABASE

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ABSTRACT

Traces of predation by drilling gastropods and peeling crabs provide important insights about predator-prey interaction in ecological as well as evolutionary times. Predation on turritelline gastropods, in this context, has been frequently discussed in literature. Here, we have estimated the intensity of predation (both drilling and peeling) on Recent turritelline gastropods from the Indian subcontinent, which has been underrepresented in previous studies. Our samples include our own collections from several Indian coasts as well as a vast collection which was locked in the archive of the Zoological Survey of India (ZSI) in Kolkata for the past 150 years. It includes samples from different parts of the Indian subcontinent as well as from many other countries. Drilling frequency (DF) of Indian turritelline species is low compared to average values of global data. We suggest that this is mainly because most of the Indian species are larger (> 4 cm) than species living elsewhere. Smaller species show higher DF and lower values of peeling frequency. Size selectivity of drill holes shows both intra- and interspecific variation. Shell thickness and ornamentation appear to be antipredatory in nature.

We have compared our results with a revised global database. Distribution of intensity of predation shows latitudinal variation where both drilling and peeling frequencies increase towards the tropics.

Key words: turritelline prey, drilling predation, peeling predation, Indian subcontinent, global dataset.

INTRODUCTION

Predation pressure exerted by a diverse group of drilling gastropods and durophagous crabs (for example, the calappids) has been identified as an important agent of selection that affects the distribution and abundance of prey community in modern marine ecosystems (Vermeij, 1977, 1987; Stanley, 2008). Traces of this kind of interaction (e.g., drill holes and repair scars) provide abundant information regarding predator-prey dynamics (Kowalewski, 2002), which can be used to test evolutionary hypotheses like enemy-driven adaptation, that is, escalation (Vermeij, 1987; Kelley & Hansen, 1993, 1996; Dietl & Alexander, 2000).

Several attempts have been made to assess tempo and mode of drilling and peeling predation since the Cretaceous (Vermeij, 1987; Allmon et al., 1990; Kowalewski et al., 1998; Walker et al., 2002; Harper, 2006), the time when many marine predators evolved and diversified (reviewed in Walker et al., 2002). These predators include the two most

diverse, modern predatory drilling gastropod families, Naticidae and Muricidae (Sohl, 1969; Taylor et al., 1983). The available methods for quantification of drilling and peeling frequencies are controversial. There are two major estimates of predation intensity. Assemblage-level study estimates overall predation intensity of the entire fossil assemblage including all, or “frequently attacked” taxa (Kowalewski, 2002). In contrast, the lower taxon frequency (LTF) estimates taxon-specific (e.g., species, genus, or family) predation intensity. Compared to assemblage-level studies, the taxon-level predation analysis is biologically and ecologically more meaningful. This is because predators may show prey preference (for example: Bardhan et al., 2012), which may be explained by cost-benefit analysis, prey availability, or prey palatability. Incorporating certain taxa which are never attacked in the analysis will make the result misleading as a measure of overall predation intensity. For this reason, the LTF may be a good way to analyze the dataset (Kowalewski, 2002). Moreover, different spe-

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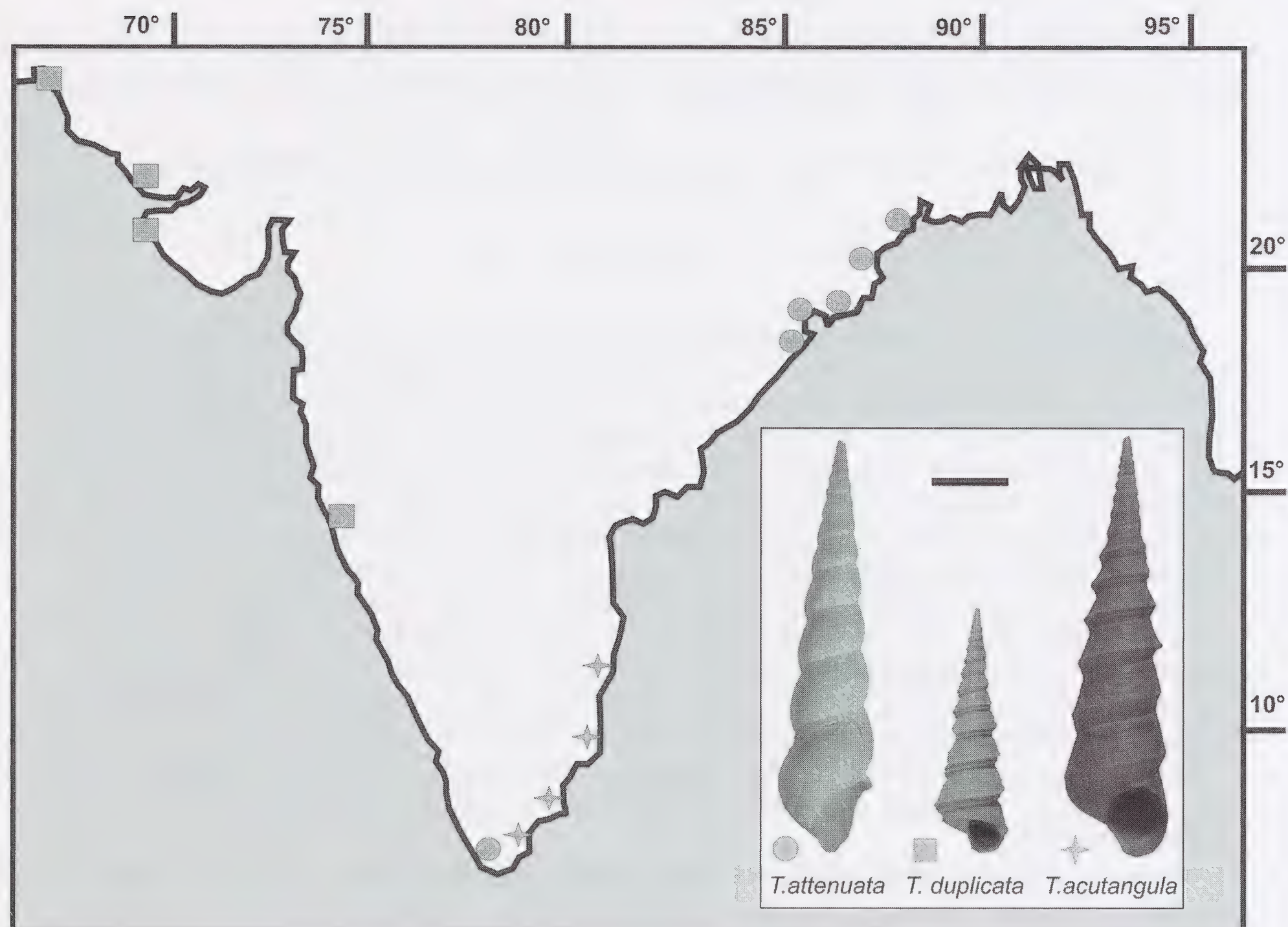


FIG. 1. Biogeographic distribution of the three abundant turrnelline species on the Indian coast (modified after Paul & Das, 2011). Scale bar = 2 cm.

cies can have differential preservation; the LTF is mostly free of this kind of taphonomic bias (Vermeij, 2002).

Turrnelline gastropods are important prey taxa for naticid drilling predation and have ubiquitous fossil record since the Cretaceous (Dudley & Vermeij, 1978; Allmon et al., 1990). It is a well-studied group and spatiotemporal distribution of naticid drilling frequency in this group has been well established (Dudley & Vermeij, 1978; Allmon et al., 1990). However, previous works on Recent turrnelline predation have been based on data mainly from the U.S.A. and western Europe (Dudley & Vermeij, 1978; Allmon et al., 1990; Gerhard et al., 1997), and Indian examples are either sparsely cited or lacking altogether. Living Indian turrnelline species are diverse ($n = 13$; Subba Rao et al., 1991, 1992; Subba Rao & Dey, 2000; Subba Rao, 2003, and personal observation). Among them the three most abundant species – that is, *Turritella attenuata* (Reeve 1849), *T. duplicata*

(Linnaeus 1758) and *T. acutangula* (Linnaeus 1758) – live allopatrically in three different coastal regions of mainland India with minor geographic overlap (Fig. 1). The present studied samples have been collected from different coasts (7,157 km coastline) along the Indian subcontinent and islands. The majority of the samples were collected by British workers before the independence of India in 1947 and are now archived in the Zoological Survey of India (ZSI), Kolkata. This collection also includes species from nearby countries (e.g., Myanmar, Sri Lanka and Pakistan). As far as we know, no data on predation on turrnellines has previously been reported from these regions. All these reasons have prompted us to use these data locked in the ZSI repository for about 150 years to make a new estimate of intensity of predation on turrnelline gastropods from Indian subcontinent, and to make a comparison with data from other parts of the world, using the LTF method.

MATERIAL AND METHODS

The material used for the present study came from two sources: (1) the collections archived in the ZSI (ZSI samples, marked as ¹ in Table 1), and (2) our own collections (marked as ² in Table 1) from different coastal regions of India, repositied in the Museum of the Department of Geological Sciences, Jadavpur University, Kolkata. ZSI samples were mainly collected by British workers before 1947 from several parts of the Indian subcontinent as well as from other parts of the world.

Old museum collections may be fraught with potential bias (discussed in Allmon, 2005). Many collections may be donated by amateurs and non-specialists, which may lead a bias towards large size and good preservation. The present ZSI collections were made by different survey parties and identified by the experts present in the ZSI (Dey, pers. comm., 2013). The experts included British malacologists who carried out systematic works on turritellines and other gastropods (e.g., Smith, 1878; Nevill, 1884; Melvill & Standen, 1889). However, we have made a thorough systematic revision of

this collections based on recent literature. Most of the species are represented by specimens of varied size and condition (intact and broken, and including corroded shells). For example, in *T. acutangula*, out of 75 specimens about 9.3% (7 specimens) and in *T. duplicata*, out of 138 specimens about 12.3% (17 specimens) shells are broken. In broken specimens, apical parts consisting of two to three whorls are missing, and in other cases outer whorls are either absent or apertural margins are broken.

Systematics of the family Turritellidae is in a state of flux (Allmon, 2011). Although the family Turritellidae is considered a monophyletic group, its phylogenetic relationship with other families within the Cerithioidea is not well understood. The subfamilies within Turritellidae are not unanimously accepted, and their monophyletic nature within the family remains unclear (Allmon, 2011). There are many genera that are not now considered as turritellines (e.g., *Mesalia*; Squires & Saul, 2007). The genus *Turritella* itself is virtually a “catch-all” genus, and many *Turritella* species have been grouped into other genera (Allmon, 2011). For our work, we have made some systematic revi-

TABLE 1. Data on drilling (DF) and peeling (PF) frequencies in living turritellines from India, Indian subcontinent, and other parts of the world. Size classes of species have been made following Dudley & Vermeij, 1978. ¹Samples are archived in the ZSI (Zoological Survey of India). Most of the locality names are mentioned in the ZSI register, but in some cases, localities are labelled as “Unknown” or “Eastern sea”. Eastern sea may refer to either east coast of India or eastern Pacific (Dey, pers. comm., 2013). ²Personal collections repositied in the Department of Geological Sciences, Jadavpur University, Kolkata. “n” denotes specimen numbers, *denotes systematic revision of the species name based on Paleobiology database (19/12/2012).

Species / Locality	Length < 4 cm			Length > 4 cm		
	n	PF	DF	n	PF	DF
<i>Turritella attenuata</i>						
Madras ¹	3	0.33	0%	20	2.85	0%
Chandipur ²	6	1	66.7%	424	1.62	18.16%
Digha ²	2	2	50%	327	2.57	11.92%
Eastern sea ¹	0	0	0%	4	8.25	0%
Unknown ¹	0	0	0%	5	6.2	0%
Cape Comaron ¹	0	0	0%	1	3	0%
Goa ¹	0	0	0%	2	2.5	0%
Pondicherry ¹	0	0	0%	2	3	0%
Tranquebar ¹	5	1.6	20%	4	1.75	0%

(continues)

(continued)

Species / Locality	Length < 4 cm			Length > 4 cm		
	n	PF	DF	n	PF	DF
<i>Turritella duplicata</i>						
Goa ¹	3	0	0%	25	1.36	0%
Okha ¹	0	0	0%	1	3	0%
Gulf of Kutch ²	74	0.72	16.21%	3	2.67	0%
Nayabazar, Bedi ¹	0	0	0%	1	1	0%
Madras ¹	3	0.67	0%	12	3.08	0%
Rangoon ¹	1	0	0%	4	1	0%
Persia ¹	33	0.55	33.3%	3	0.67	33.3%
Baluchistan ¹	51	0.37	9.8%	0	0	0%
Dwarka ²	35	0.69	34.3%	7	1.28	0%
Ceylon ¹	0	0	0%	1	1	0%
<i>Turritella acutangula</i>						
Madras ¹	20	0.1	5%	15	1.33	6.67%
Pondicherry ¹	1	0	0%	9	1	0%
Mallipattinam ¹	0	0	0%	2	2	0%
Arabian coast	0	0	0%	2	2	0%
Kakinada ¹	0	0	0%	2	3	0%
Rameswaram ¹	0	0	0%	3	1	0%
Gulf of Mayanmar	1	0	0%	2	1	0%
Goa ¹	0	0	0%	8	1.25	0%
Chilka Lake	3	0	33.3%	2	0.5	0%
Tutikorin ¹	5	0	40%	0	0	0%
<i>Turritella bacillum</i>						
Rangoon ¹	2	0.5	0%	0	0	0%
<i>Turritella terebra</i>						
Eastern Sea ¹	0	0	0%	5	4	0%
<i>Turritella columnaris</i>						
Ceylon ¹	4	2.5	25%	4	6.5	50%
Okha ¹	1	0	0%	6	1.83	16.7%
Unknown ¹	30	0.4	20%	2	3	0%
<i>Turritella (Haustator) trisulcata</i>						
Tamilnadu ¹	19	0.16	15.7%	0	0	0%
Subramannium temple ¹	10	0.3	30%	0	0	0%
Baluchistan ¹	7	1.14	28.6%	3	2	0%
<i>Turritella nebulosa</i>						
Madras ¹	0	0	0%	1	0	100%
<i>Turritella capensis</i>						
False Bay (Andhra) ¹	4	0.5	0%	0	0	0%

(continues)

(continued)

Species / Locality	Length < 4 cm			Length > 4 cm		
	n	PF	DF	n	PF	DF
Cape of Good Hope ¹	6	0.33	0%	0	0	0%
False Bay ¹	10	0.3	20%	0	0	0%
<i>Turritella fascialis</i>						
Muscat ¹	14	0.21	35.7%	0	0	0%
Gulf of Oman ¹	12	0	41.7%	0	0	0%
<i>Turritella vitullata</i>						
China ¹	7	0.43	0%	0	0	0%
Persian Gulf ¹	6	1	0%	4	1.25	25%
<i>Colpospira sinuata</i> *						
Australia ¹	9	0.89	22.2%	0	0	0%
Unknown ¹	5	0.6	40%	0	0	0%
<i>Turritella carinifera</i>						
Cape of Good Hope ¹	13	0.77	7.7%	4	4	0%
<i>Turritella nivea</i>						
Baluchistan ¹	22	0.28	13.6%	1	1	0%
Persian Gulf ¹	6	0	0%	1	0	0%
<i>Archimediella fastigiata</i> *						
Andaman ¹	5	0.4	0%	2	1.5	50%
Margui Archipelago ¹	2	1	50%	0	0	0%
<i>Turritella infraconstricta</i>						
Andaman ¹	5	0.8	0%	1	1	0%
<i>Turritella (Haustator) monilifera</i>						
Andaman ¹	0	0	0%	1	3	0%
<i>Maoricolpus rosea</i> *						
New Zealand ¹	6	1.67	0%	6	1.5	0%
<i>Turritella variegatus</i>						
West Indies ¹	0	0	0%	5	3.6	0%
<i>Turritella fuscocincta</i>						
Australia ¹	31	0.26	0%	0	0	0%
<i>Turritella hanleyana</i>						
Ceylon ¹	0	0	0%	1	9	0%
<i>Turritella communis</i>						
England ¹	4	2	0%	4	2.75	0
<i>Turritella exoleata</i>						
West Indies ¹	18	0.5	0%	0	0	0%
<i>Archimediella maculata</i> *						
Aden ¹	5	1.8	20%	3	1.33	66.7%

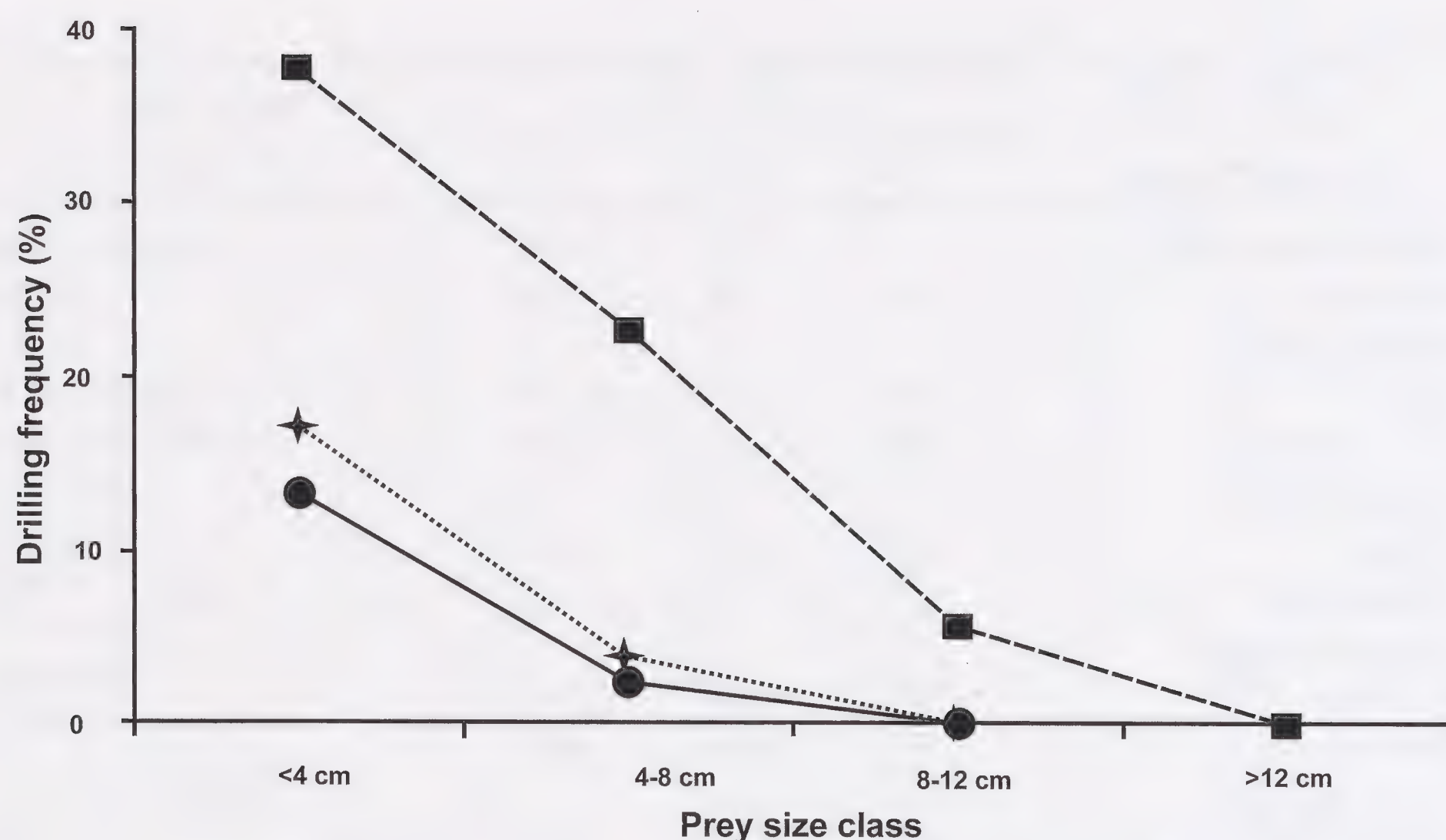


FIG. 2. Frequency of naticid drill holes for four different size classes in three abundant Indian species. Note a fall in DF during ontogeny of all species. Symbols: squares: *T. attenuata*, stars: *T. duplicata*, circles: *T. acutangula*.

sion of some species, based on consultation with taxa listed in the Paleobiology Database (www.pbdb.org). For example, one species that has been labelled as *Turritella fastigiata* in the ZSI collections has now been designated as *Archimediella fastigiata* (for other revised species: Table 1). Identification of most of the present turritelline species are based on recent monographs published by Zoological Survey of India (Subba Rao et al., 1991, 1992; Subba Rao & Dey, 2000; Subba Rao, 2003). The three main Indian species, namely, *T. attenuata*, *T. duplicata* and *T. acutangula*, are taxonomically stable and have been reported also from other parts of the world (Allmon, 2011; Waite & Strasser, 2011). *Turritella capensis* has been previously wrongly labelled as *T. "knyonaensis"*. We have now corrected it in the register of ZSI collection.

Although there is no way to know with certainty what biases affected the collecting represented by the ZSI specimens, circumstantial information suggests that we may use them for present purposes with some confidence. For example, the distribution of smaller and larger size classes in both ZSI and our new collections are comparable, which indicates that the ZSI collections for *T. attenuata* are not biased towards larger size classes. Many ZSI species

show high frequencies of drilling or peeling (Table 1). For example, drilling frequency or DF, a measure of how frequently a species has been drilled by gastropods, on *Archimediella fastigiata* and peeling frequency or PF, a measure of how frequently a species has been peeled by crabs and subsequently repaired, on *T. duplicata* in the ZSI collection are both high, suggesting that drilled or peeled specimens were not excluded from ZSI collection.

In addition, the present study includes only 576 ZSI specimens (40% of the total specimens considered), and data from these specimens may show substantial differences from specimens in our own collection. For example, *T. attenuata* collected by us has DF which varies between 50%–67% in smaller specimens, whereas in ZSI collection, DF varies from around 0%–20% in the same size class. This may be the artefact of low sample size of ZSI collections (Table 1). When sample sizes are large (e.g., *T. duplicata* from Persia, $n = 33$ in ZSI collection and Dwarka, $n = 35$ and Kutch, $n = 74$ in our own collection), drilling frequencies are very similar (Table 1). We do not claim that DFs are invariant in a spatial framework; indeed, previous work has shown that a species may show spatial variability in predation intensity (Leighton, 1999; Dietl & Kelley, 2001;

Harries & Schopf, 2007; Martinelli et al., 2013). Therefore, we argue that, at least for our purposes, although old museum collections are potentially biased, they are usually better than nothing, especially for completely unreported data in a neglected region of the world, such as India (Allmon, 2005; pers. comm., 2012).

Our own collections were made from several localities along the eastern (Chandipur, Orissa; Digha, West Bengal; $n = 759$) and western coasts (Gulf of Kutch and Dwarka, Gujarat; $n = 119$) of India (Fig. 1), from two consecutive field trips during 2010 and 2011. These have enabled us to study spatial variation of predation on modern turritelline clade for which predation data are available from other areas. About 1,454 complete or nearly complete shells, including the ZSI material, were examined to record two types of predation marks (drill holes and repair scars). We have also compared our present data with a revised global data set (taken mainly from Buchanan, 1958; Dudley & Vermeij, 1978; Tull & Bohning-Gaese, 1993; Gerhard et al., 1997; Sawyer & Zuschin, 2010). Since predation frequency varies with prey size, we have measured it for different size classes. For the global dataset, only two size categories were compared – < 4 cm versus > 4 cm (Table 1). For three most abundant Indian species, detailed analyses were done by classifying them in four size-categories (i.e., < 4 cm, 4–8 cm, 8–12 cm and > 12 cm) (Fig. 2). All detailed studies on various aspects of drilling predation, including predator-prey behavioural data (e.g., size and site selectivity), were only made for the three abundant Indian species ($n = 1,106$). Otherwise, all samples were pooled together to identify spatial trends at global scale.

The majority of drill holes on the studied turritelline species are “truncated spherical paraboloids”, which are typical of those made by naticid gastropods (Carriker & Yochelson, 1968; Carriker, 1981). Drilling frequency has been calculated as percentage of shells with at least one complete drill hole divided by the total number of shells (N) (Allmon et al., 1990). Unsuccessful and multiple drill holes speak for prey effectiveness against predation. Frequency of unsuccessful drill holes has been defined as a ratio between total incomplete drill holes and total number of attempted holes, while frequency of multiple drill holes has been defined as number of drill holes which are found in multiple drilled specimens divided by the total number of attempted holes (Vermeij, 1987; Kelley et al., 2001). For peeling

predation, we have concentrated on repaired peeling scars only. Predators can attack turritelline gastropods in different ways: some of them will break the spire, some completely crush the shell, and some ingest the prey whole (Allmon, 2011). These activities are almost impossible to track in dead turritelline shells. In comparison, unsuccessful apertural peeling by calappid crabs, as indicated by repair scars, is relatively convenient to trace and quantify. Aperture-parallel repair scars, which represent minor lip breakage, have been excluded from the quantification of peeling predation (Vermeij & Dudley, 1982), as these types of breaks can also be caused by physical agents. PF has been calculated as the total number of repair scars divided by the total number of shells examined, expressed in decimal values (Allmon et al., 1990).

In order to understand predator behaviour (i.e., size- and site-selectivity), the following observations have been made: relationship between outer bore hole diameter (OBD, which tells about size of the predator involved; Kitchell et al., 1986) and prey size (plot of OBD against prey length); and vertical and radial positions of the drill holes with respect to shell base and apertural plane, and also the location of holes on the whorl. Our null hypothesis was that the drill holes are distributed randomly, both vertically and radially. Prey size was measured in two different ways: length from apex to the base of aperture (L), and the maximum whorl diameter (D), measured at last whorl.

Previous studies on turritellines have attempted to address how specific prey adaptations can influence predation (Signor, 1985; Allmon et al., 1990; Tull & Bohning-Gaese, 1993). In general, it would be expected that robust and ornamented forms are relatively more resistant than less sculptured forms. Allmon et al. (1990) used a qualitative measure of shell ornamentation to rank them in different defensive categories. We have developed a similar, more quantitative rather than qualitative ornamentation scheme, based on strength and number of ribs present on these species (Table 2). Based on it, we have put all turritelline species into four different ornamentation classes. If a species has only weak ribs, we have counted the number of these weak ribs to classify them into different ornamentation classes. For example, category 1 has no strong ribs; it has less than/equal to five weak ribs. Any species which have only one strong rib or five to eight weak ribs will be ranked as category 2. This scheme

TABLE 2. Classification of different turritelline species of Indian subcontinent based on their strength of ornamentation. Relationship between predation frequencies (DF and PF) with ornamentation index is also shown. See text for details.

Ornamentation Index	Number of strong ribs	Number of weak ribs	N	DF	PF
Class 1	0	≤ 5	4	25%	2.5
Class 2	1	5-8	801	15.23%	2.07
Class 3	2	1–2	307	14.66%	0.94
Class 4	> 2	> 3	154	10.39%	0.73

gives maximum weighting to number of strong ribs and then number of weak ribs present on shells. Shell slenderness (i.e., high L to D ratio) also appears to influence predation, either because slender species are difficult to detect and capture (Allmon et al., 1990), or because their smaller aperture size discourages peeling predators (Signor, 1985). In addition, we have measured shell thickness (T) in two ways. Firstly, at different growth stages (by breaking the shell), to document ontogenetic variation; secondly, at the apertural margin.

Because most of the specimens considered here were likely collected dead, taphonomic processes may have biased the samples; drilled shell may behave differently during transportation and may be preferentially broken (Dudley & Vermeij, 1978; Roy et al., 1994; but see Hagstrom, 1996; Kaplan & Baumiller, 2000; Zuschin & Stanton, 2001). On the other hand, drilled shells may be better preserved because a naticid takes its prey within the sediment before drilling (Edwards, 1974). Hermit crabs can also alter general trends of taphonomic preservation of shells since they accept both infaunal and epifaunal gastropod shells. Because of these complexities, we have assumed that the drilled and undrilled shells have equal preservation potential, following others (Dudley & Vermeij, 1978; Vermeij & Dudley, 1982; Allmon et al., 1990; Tull & Bohning-Gaese, 1993; Hagadorn & Boyajian, 1997).

RESULTS

Predation on Indian Subcontinental Species

Drilling Predation

Drilling Frequency: Among the three most abundant Indian species, *T. attenuata* shows

the highest value of DF (15.23%, n = 801), which is not significantly different from *T. duplicata* (13.47%, n = 230, p = 0.5081). In contrast, DF on *T. acutangula* is very low (6.67%, n = 75) (Table 1). Ontogenetic variation in DF is also evident in all three species; smaller individuals (< 4 cm in length) show higher DF and it decreases as the prey body size increases (Table 1, Fig. 2).

Data from ZSI and our collections, representing 14 turritelline species from the Indian subcontinent, show overall DF of 14.24% (n = 1,222). Ontogenetic variation in DF is not evident, smaller individuals (< 4 cm) show DF of 16.36% (n = 324), which is comparable with DF for larger counterpart (> 4 cm) 13.47% (n = 898; p = 0.2019) (Table 1).

Frequencies of incomplete drill holes also show insignificant variability. For *T. attenuata*, *T. duplicata* and *T. acutangula*, the values are 2.58%, 2.17%, and 3.08% respectively. Multiple drill holes are rare for *T. duplicata* (0.65%) and *T. attenuata* (0.25%); no instance of multiple drill holes has been found in *T. acutangula*.

Within-species spatial variations in DF for turritellines are also evident. For example, *T. attenuata* in Digha (21°41'N, 87°33'E) shows overall drilling frequency of 12.16% (n = 329), while in Chandipur (21°28'N, 87°01'E) it is 18.83% (n = 430) (p = 0.0129). Similarly, for *T. duplicata*, drilling frequency varies from 9.8% (n = 51) at Baluchisthan (28°53'N, 64°25'E) to 28.57% (n = 42) at Dwarka (22°14'N, 68°58'E) (Table 1) (p = 0.0198).

Predator Behaviour: When size stereotypy of predation was studied by plotting predator's size (i.e., OBD) against prey shell length, *T. attenuata* shows overall poor correlation (r² = 0.003, p << 0.005) (Fig. 3A), but the sample from Digha shows relatively good correlation (r² = 0.285). In the case of *T. duplicata*, this relation

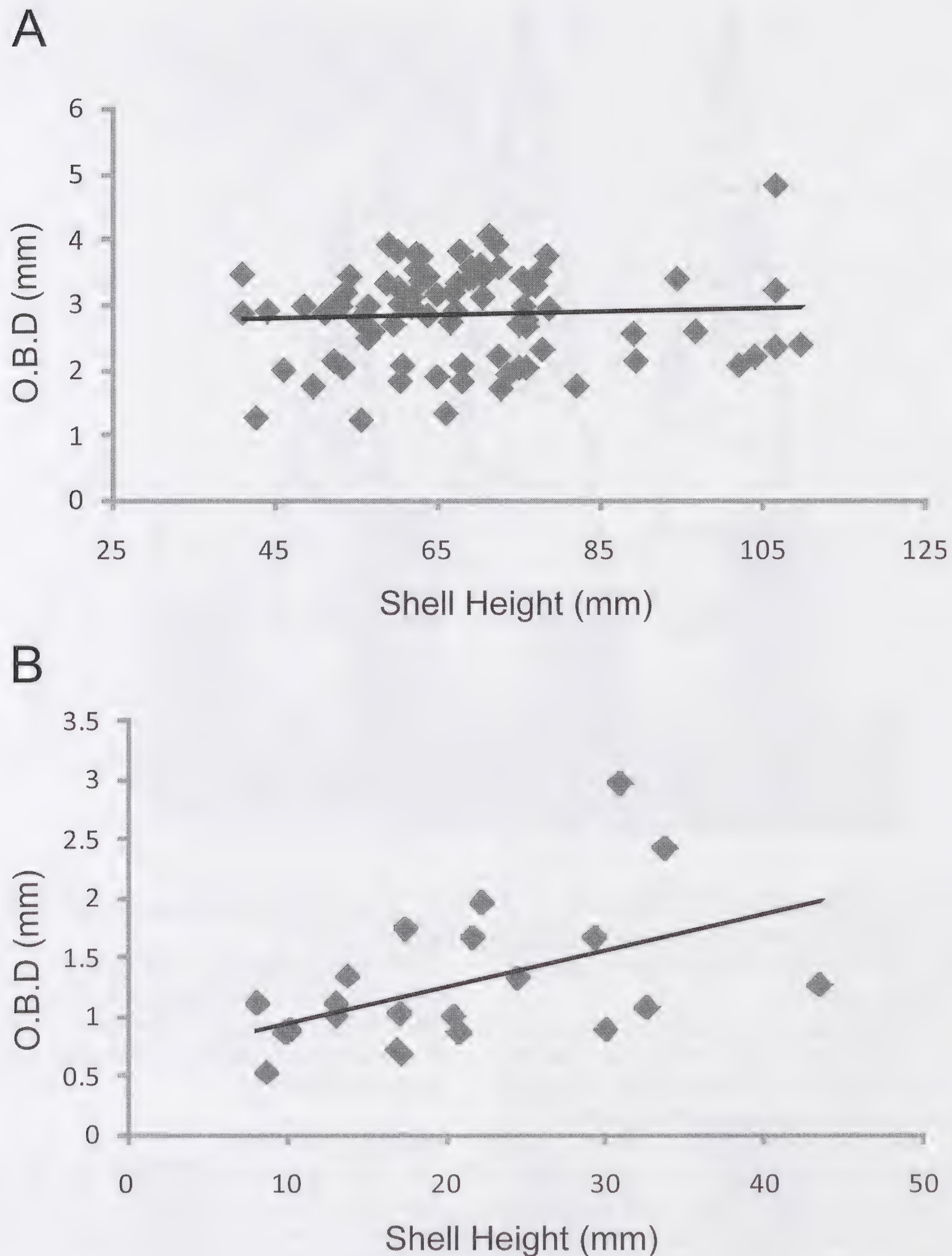


FIG. 3. Relationship between outer borehole diameter (OBD, proxy for predator size) of naticid drill holes and prey shell height for *T. attenuata* (3A); $y = 0.002x + 2.579$ (n = 87, $r^2 = 0.003$, $p = 7.791E-22$) and *T. duplicata* (3B); $y = 0.030x + 0.630$ (n = 22, $r^2 = 0.238$, $p = 1.433E-08$).

holds true ($r^2 = 0.238$, $p = 0.0115$) (Fig. 3B). We do not have enough samples for *T. acutangula* to show such correlation.

Predator site selectivity for drilling was studied by looking at distributions of drill holes on the prey shell, both vertically and radially. In vertical profile, drill holes are highly stereo-

typed; that is, mostly located on the last two whorls away from the aperture in *T. attenuata*, while, for *T. duplicata*, holes are randomly (no preferential site) distributed throughout the shell (Fig. 4). In a radial quadrant system, drill holes on both *T. attenuata* and *T. duplicata* are equally distributed in both apertural (I and

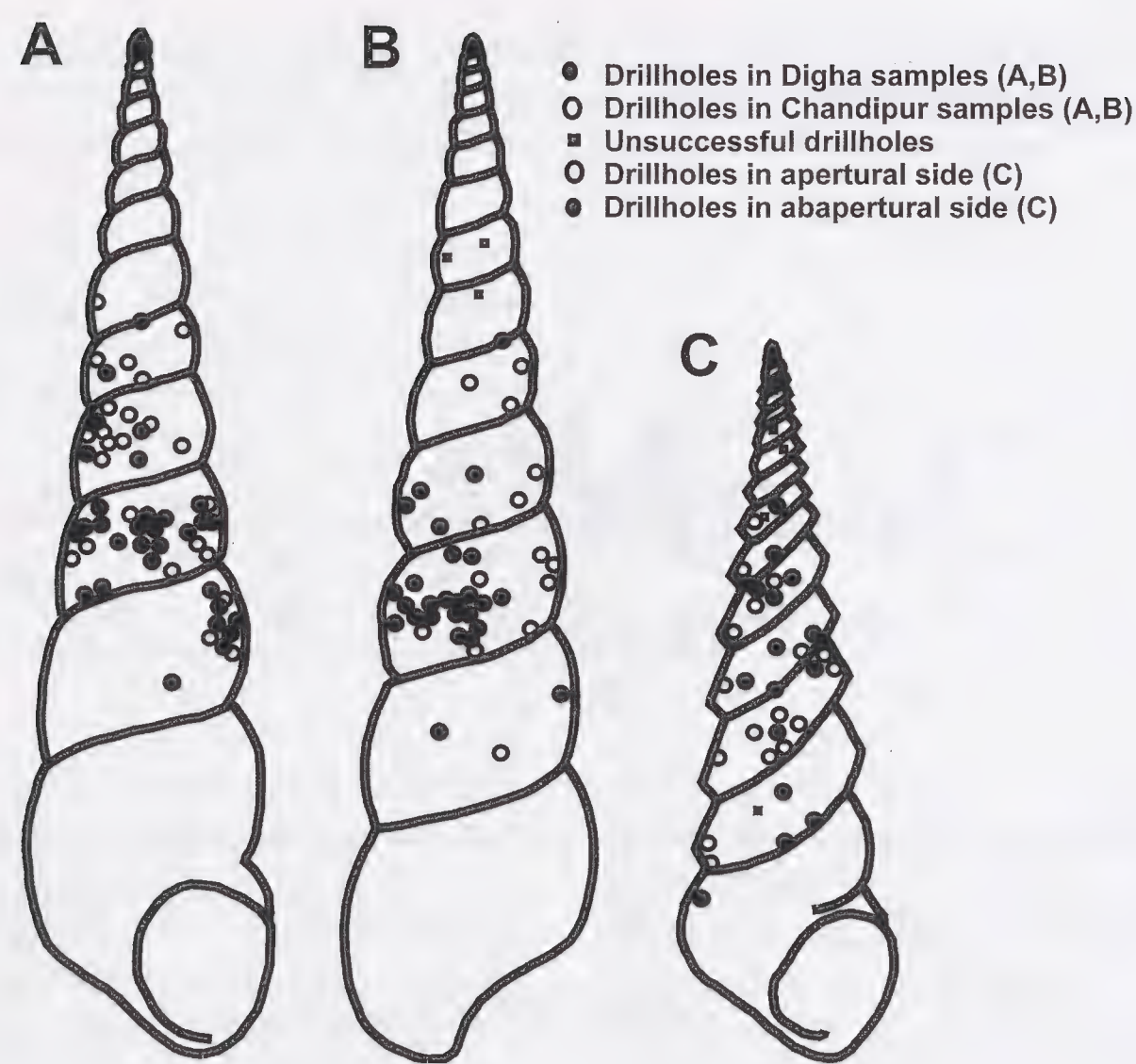


FIG. 4. Schematic diagrams showing vertical position of drill holes on apertural (A) and abapertural (B) sides of *T. attenuata* (n = 103) from different localities. For *T. duplicata* (n = 40) apertural and abapertural drill holes are represented in a single figure (C). Symbols in the inset provide drill hole information for different sides.

IV) and abapertural (II and III) sides (Fig. 5). *T. acutangula* has low drilling frequency (only five shells are drilled) and therefore the stereotype has not been studied.

Drill hole (both successful and unsuccessful) positions on the whorl (360° segment of a helically coiled shell) were studied to see whether they were located on the suture or whorl (Fig. 4). For *T. attenuata*, 77.5% of successful holes were located near the mid-whorl and 22.5% were located on the suture or close to it. Similarly, for *T. duplicata*, 71.8% successful holes were located near the mid-whorl, and 28.2% were located near or at the suture. Unsuccessful holes are more common on *T. attenuata*, with 66.67% located on the whorl. The majority (72.22%) of unsuccessful holes, however, are located beyond the stereotyped area (where most of the successful holes are located), that is, towards the apex. In *T. duplicata*, unsuccessful drill holes (n = 5) are found on the whorl as well as on the suture.

Anti-Drilling Prey Features: For *T. attenuata* and *T. acutangula*, shell thickness increases with ontogeny (Fig. 6, $r^2 = 0.7044$ and 0.8529 , respectively). In *T. duplicata*, shell thickness (r^2

$= 0.0052$) does not change significantly during growth (Fig. 6).

The three species were categorized on the strength of ornamentation (Table 2): *T. attenuata* belongs to class 2, whereas *T. duplicata* and *T. acutangula* have been classified into class 3 and 4 respectively.

Peeling Predation

Peeling Frequency: PF is highly variable, from species to species, as well as with ontogeny within a species. *Turritella attenuata* shows a maximum PF of 2.07 (n = 801), while two other species show relatively low frequencies of peeling (0.81 for *T. acutangula* and 0.9 for *T. duplicata*) (Table 1).

The overall PF from the Indian subcontinent, incorporating all 14 species together, shows a value of 1.62 (n = 1,222). Smaller specimens (< 4 cm) have a lower PF (0.5, n = 324), compared to the larger shells (2.02, n = 898) (Table 1). This type of size dependence is also evident for the three most abundant species.

Peeling frequency for a single species also varies spatially (Table 1). For example, *T. attenuata* from Chandipur shows PF of 1.61 (n =

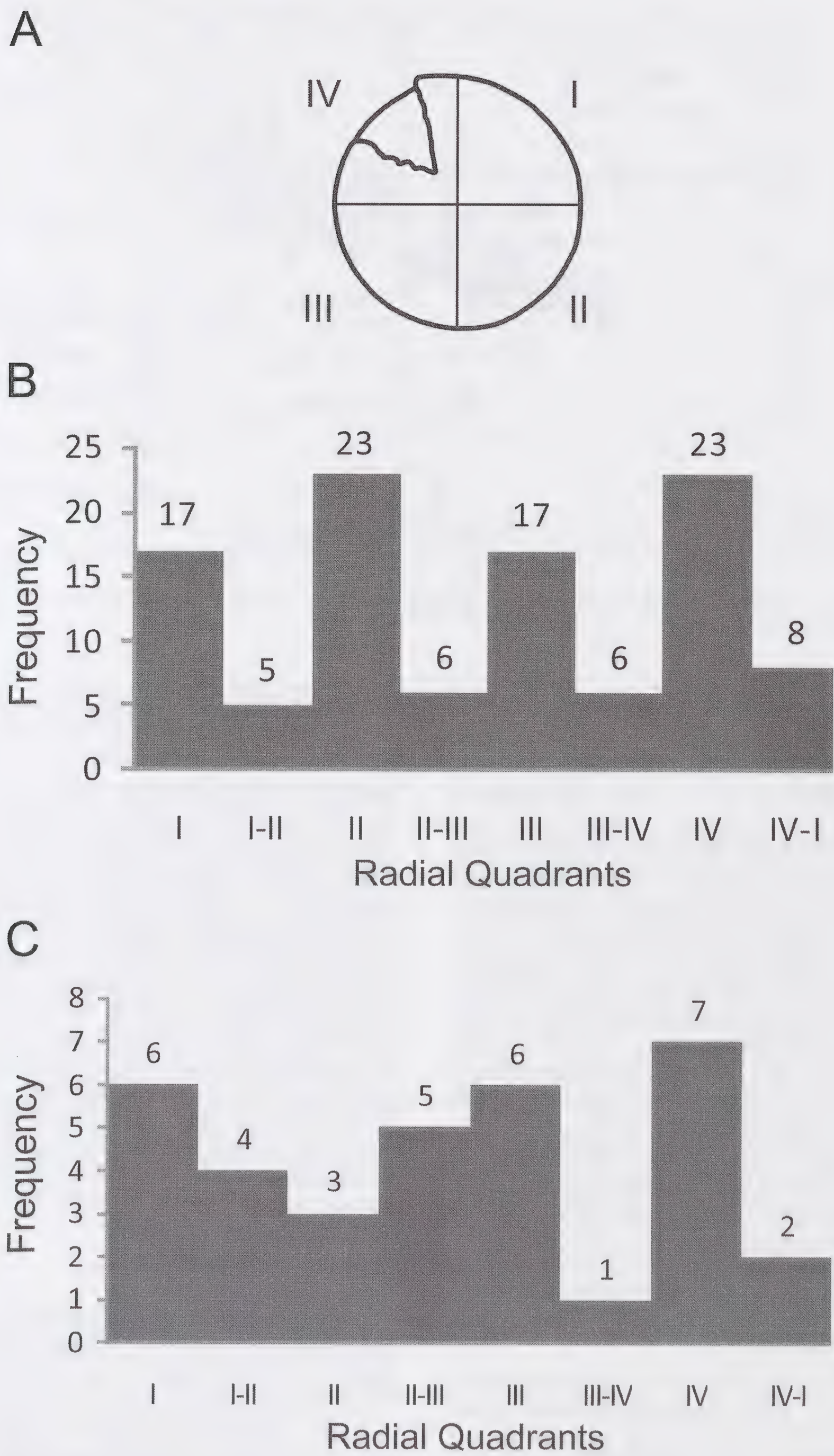


FIG. 5. A) shows the base of a turritelline shell indicating the distribution of different quadrants in radial system (after Allmon et al., 1990), B) and C) show radial distribution of drill holes on intact shells of *T. attenuata* (n = 105) and *T. duplicata* (n = 34) respectively.

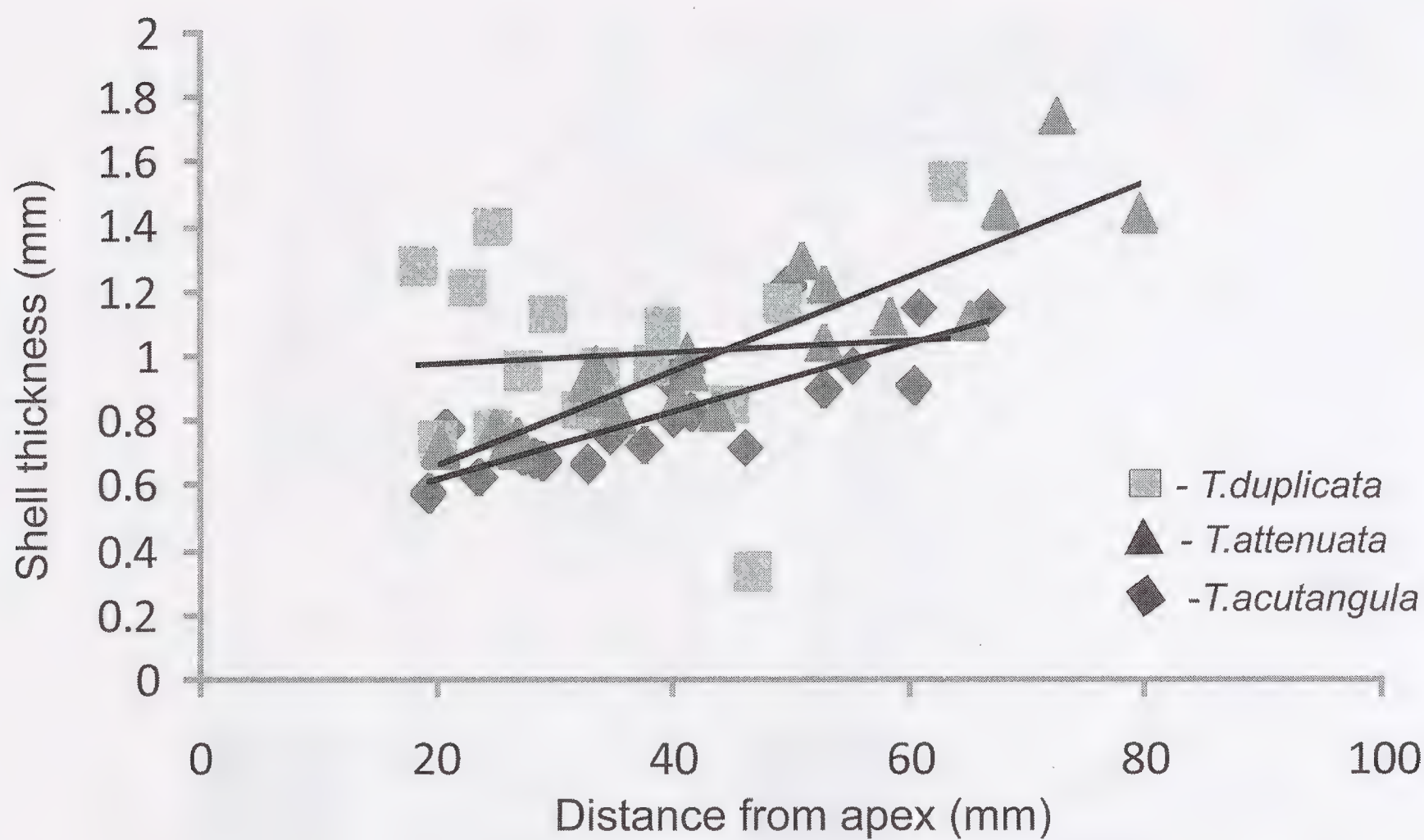


FIG. 6. Variation of shell thickness at different distances from the apex of the three abundant Indian species.

430), while samples from Digha show a value as high as 2.57 (n = 329) (p = 0.0011). Similarly, for *T. duplicata*, PF varies from 0.37 (n = 51) in Baluchistan to 0.76 (n = 42) at Dwarka (p = 0.0002).

Anti-Peeling Prey Features: Recent turritelline species show wide interspecific diversity of shell geometry (Allmon, 2011). Slenderness (ratio of shell diameter, D, to shell length, L) can be used to group them into different categories. Even at a similar size, some species are more slender (e.g. *T. columnaris*; D/L = 0.22), and others are inflated (e.g., *T. duplicata*; D/L = 0.39). It is seen that PF decreases with an increase in D/L values i.e., inflated species are more resistant to peeling (Fig. 7).

Mean shell thickness at the aperture, normalized by maximum whorl diameter, for the three abundant Indian species does not vary signifi-

cantly (p = 0.5) (Table 3), but PF varies significantly among them. This suggests that greater shell thickness at the aperture is not effective against peeling predation. The persistence of a thin shell at the aperture may be a result of morphological constraint (Seilacher, 1970; Gould, 1980). It indicates that, thin aperture in turritellines is inherited from ancestors and a symplesiomorphic character of the family (see also Marwick & Hutt, 1957). But ornamental strength (definition: Table 2) varies greatly within turritelline species and has a negative correlation with peeling intensity. According to our ornamentation scheme, *T. attenuata* belongs to class 2 and has an overall peeling frequency of 2.07 (n = 801), whereas *T. duplicata* (class 3) and *T. acutangula* (class 4) have overall peeling frequencies of 0.9 (n = 230) and 0.81 (n = 75) respectively (p = 0.0387) (Table 2).

TABLE 3. Mean apertural thickness, normalized by maximum whorl diameter (MWD), of the three abundant Indian species.

Species	Mean apertural thickness / MWD
<i>T. attenuata</i>	0.0272
<i>T. duplicata</i>	0.0307
<i>T. acutangula</i>	0.0237

Global Predation Trends

Recent turritelline species are diverse and distributed globally (Allmon, 2011). We combined data from the literature and the collections discussed here to make a new global dataset to track the global trends of naticid predation and crab peeling on turritelline gastropods. The analyses of these data reveal that overall DF of all Recent turritellines is 32.55% (n = 5,950; Buchanan, 1958; Dudley & Vermeij, 1978; Tull

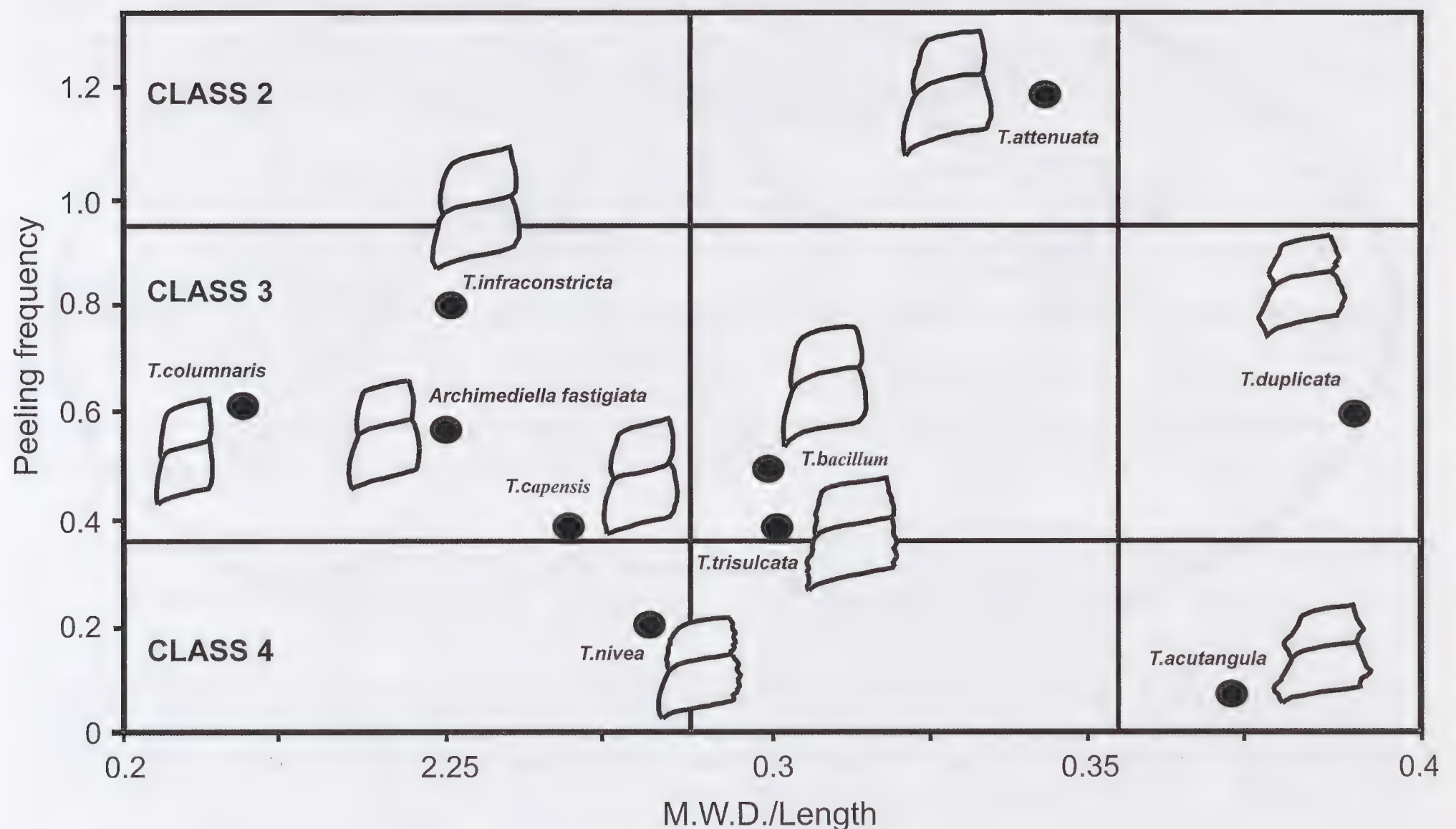


FIG. 7. Relationship among peeling frequency (PF), shell geometry and ornamental strength (subjectively ranked into different classes; for details see text).

& Bohning-Gaese, 1993; Sawyer & Zuschin, 2010; present data), and overall PF is 1.47 ($n = 1,561$; Gerhard et al., 1997; present data). For detail, see Appendix 1. Drilling and peeling frequencies vary in different size classes. Larger specimens (> 4 cm) show drilling and peeling frequencies of 18.43% ($n = 1,595$) and 2.09 ($n = 945$), respectively, whereas smaller specimens (< 4 cm) show 22.18% ($n = 1,709$) and 0.54 ($n = 508$), respectively (Dudley & Vermeij, 1978; Tull & Bohning-Gaese, 1993, Gerhard et al., 1997; present data). We have excluded some references from this analysis because of the lack of size data (e.g., Buchanan, 1958; Sawyer & Zuschin, 2010).

DISCUSSION

The new data presented here, along with other recent literature (Dudley & Vermeij, 1978; Allmon et al., 1990; Tull & Bohning-Gaese, 1993; Hagadorn & Boyajian, 1997), has prompted us to expand the documentation, analysis and interpretation of drilling and peeling predation on Recent turritelline gastropods throughout the world.

Allmon et al. (1990) stated that functional analyses of turritelline shells are difficult and

challenging. Some of the species have active defensive responses (Allmon, 2011), and some show behavioral avoidance of predators (Allmon et al., 1990). Predator-prey interaction can also be underestimated or overestimated due to such other biotic factors as predator availability, prey avoidance, prey preference, and diversity and the abundance of both predators and prey. The nature of naticid predation such as multiple strategies against prey (e.g., killing a prey species either by drilling or by smothering, as observed in several species of the genus *Polinices*; Vermeij, 1980; Ansell & Morton, 1985, 1987, Kabat, 1990) and fish predation by either crushing or swallowing (Dudley & Vermeij, 1978; Zipser & Vermeij, 1978) may also affect the nature of the record of predation. Our very large sample size reveals some interesting aspects of turritelline predation which will be discussed here.

Drilling Predation

Drilling Frequency

The average drilling frequency on all Recent turritelline gastropods from the Indian subcontinent is 14.24% ($n = 1,222$) which is not significantly different from the DF when only the three most abundant Indian species

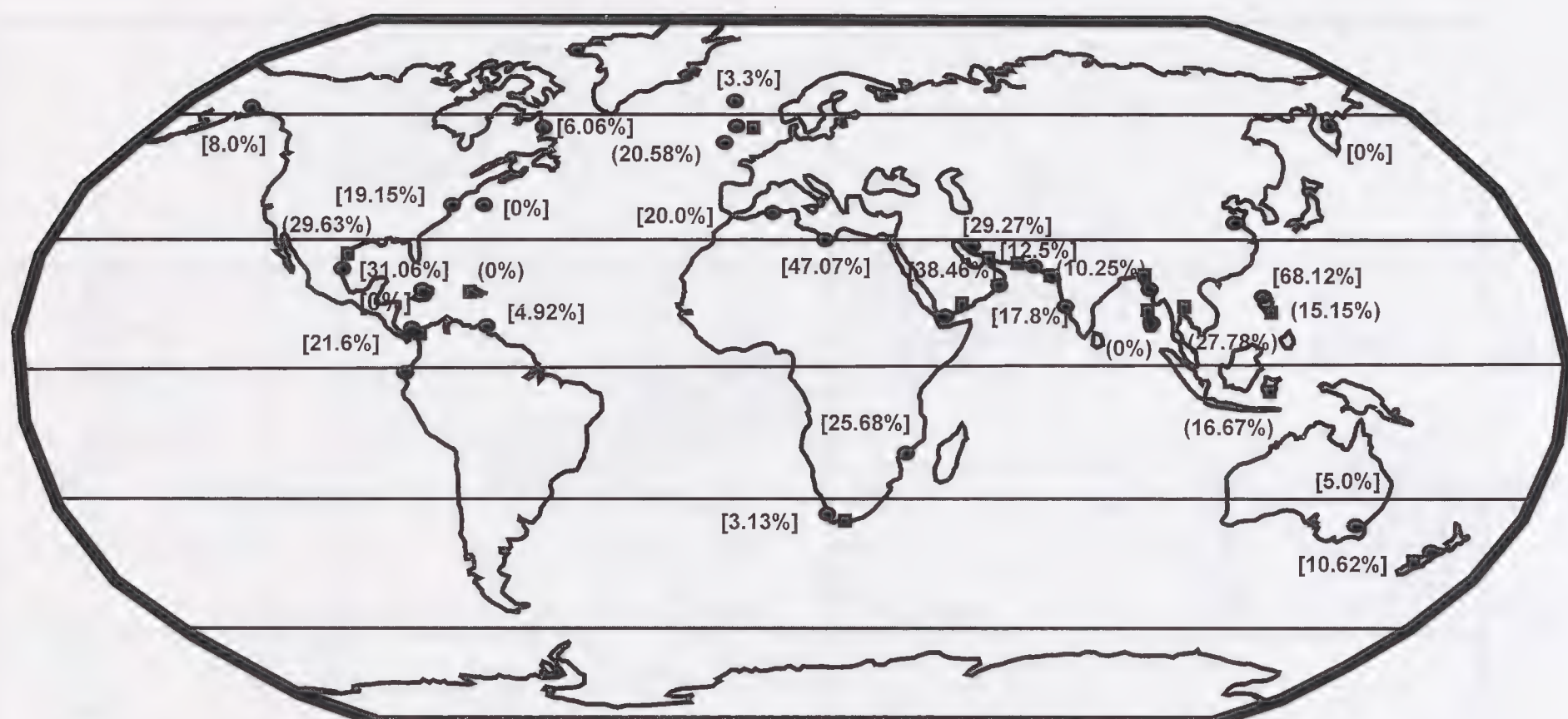


FIG. 8. Geographic distribution of drilling predation on turritelline gastropods. Circles represent smaller samples and squares represent larger samples. Drilling percentages for localities having specimen numbers more than 10 are enclosed within square brackets, smaller samples in parentheses. For the rest only symbols are plotted.

(14.69%, $n = 1,137$; $p = 0.7561$) were considered. Both values are low with respect to the DF for all turritelline gastropods worldwide, which is 32.55% ($n = 5,950$; $p < 0.0001$). One conspicuous trend in predation on turritelline gastropods is that predation frequency varies with prey size classes (Dudley & Vermeij, 1978). For drilling predation, DF decreases with increase in prey shell size for each of the three most abundant Indian species (18.18% for shells below 4 cm and 13.24% for shells above 4 cm; see Table 1). Even further subdivision of size distribution (e.g. < 4 cm, 4–8 cm, 8–12 cm, and > 12 cm) shows the same trend (Fig. 2). Drilling frequencies drop drastically above 4 cm. Smaller individuals (< 4 cm) of all Indian subcontinental species show relatively higher drilling frequency (16.36%) compared to the larger size class (13.47%) (Table 1; Tull & Bohning-Gaese, 1993). This observation is consistent with the idea that larger individuals are relatively more resistant against drilling predation (Dudley & Vermeij, 1978; Allmon et al., 1990; Tull & Bohning-Gaese, 1993). Within the Indian subcontinent, out of the 14 species studied, only three species (21%) have adult body sizes smaller than 4 cm. It may explain why the Indian population shows a relatively low drilling frequency compared to the rest of the world, of which, out of 31 species (whose drilling data are known; Table 1; Dudley & Vermeij, 1978), 19 species (61%) are smaller than 4 cm.

A higher DF in smaller specimens is also evident in previous studies of the fossil record (Dudley & Vermeij, 1978; Arua & Haque, 1989; Allmon et al., 1990; Tull & Bohning-Gaese, 1993; Hagadorn & Boyajian, 1997). The higher drilling frequency in smaller specimens may be due to several reasons; naticids may target young individuals for easy manipulation of prey, or it may simply be a change in the life mode of the prey during ontogeny. Waite & Strasser (2011) suggested that the smaller individuals of *T. duplicata* (mainly below 4 cm length) may live infaunally, while the larger individuals may be more frequently on top of the sediment surface; this might result in more predation on juveniles since naticid predators generally target infaunal prey (Sawyer & Zuschin, 2010).

Despite having the same shell morphology and size classes, some individual species show geographic variation in DF. Drilling frequency also varies for different species from different areas (Allmon et al., 1990). Among the three abundant Indian species, *T. attenuata* shows significant variation within the different areas of the eastern coast. The Digha population has drilling frequency of 12.16% ($n = 329$) while the Chandipur population occurring about 80 km south has 18.83% DF ($n = 430$; $p = 0.0129$). One possible reason may be that naticid are both abundant and diverse in Chandipur (Subba Rao et al., 1991) and frequently drill other prey taxa (Mondal et al., 2010). But if different populations of the same species are

TABLE 4. Latitudinal variation of predation frequencies (DF and PF) based on global database. Numbers within parentheses indicate specimen numbers. See text for details. Note that data for PF from 60°–70° are not available. Sources: Buchanan, 1958; Dudley & Vermeij, 1978; Tull & Bohning-Gaese, 1993; Gerhard et al., 1997; Sawyer & Zuschin, 2010; present data.

Predation frequencies	Latitudes						
	0°–10°	10°–20°	20°–30°	30°–40°	40°–50°	50°–60°	60°–70°
DF	55.7% (521)	22.3% (2,103)	22.3% (341)	12.8% (390)	10.1% (119)	0% (28)	6.7% (105)
PF	3.21 (14)	1.85 (985)	0.59 (118)	0.83 (210)	1.58 (12)	2.83 (8)	-

geographically widespread, they may show more latitudinal variation. For *T. duplicata*, drilling frequency varies from 9.8% (n = 51) at Baluchistan to 28.57% (n = 42; p = 0.0198) at Dwarka, which is about 2,358 km south of the former and is closer to the subtropics. Similar latitudinal differences may be seen when the global data are pooled for the all Recent turritelline species (Fig. 8, Table 4).

Predator Behavior

The behavior of predators is important to understand the dynamics of predator-prey interaction. Numerous studies of naticid gastropods have revealed that their predatory behaviour has evolved with time. Prey handling, prey manipulation, drilling site selection, prey preference, etc. have become more efficient (Vermeij, 1983; Kelley & Hansen, 1993, 1996; Dietl & Alexander, 2000). The new data set discussed here may allow investigation of these parameters.

Preference of vertical location for drilling reflects predator’s efficiency to drill on the thinnest part of the shell (Kitchell, 1986). *Turritella attenuata* shows stereotyped site selection. Most of the drill holes are concentrated at 50%–60% of the shell length from the apex, that is, two whorls away from the aperture (Fig. 4). Allmon et al. (1990) and Hagadorn & Boyajian (1997) also found similar site selectivity in fossil turritelline species. Allmon et al. (1990) argued that this site preference is related to predator’s behaviour of choosing the thinnest part of the shell, but did not provide any quantification for thickness. Among the three abundant species, we did not find any support for this generalization. For two species (*T. attenuata* and *T. acutangula*), shell thickness is positively correlated with ontogeny (Fig. 6), and it appears that the preferred site is not the thinnest part of the shell. This observation is not consistent with the prediction of the general cost-benefit theory

(Kitchell, 1986) and needs further attention. In *T. duplicata*, shell thickness does not change significantly during ontogeny which may explain the lack of drill hole site stereotypy (Fig. 6).

Radial location of drill holes is another measure of predatory selectivity because it indicates whether the naticids attempt to drill into the apertural or abapertural side of the prey shell (Adegoke & Tevesz, 1974). For turritellines, quadrants II and III (Fig. 5) will be mostly targeted when the prey crawls above the sediment. Quadrant I and II will be targeted when the prey is stationary in its infaunal live position (Allmon, 2011; Waite & Strasser, 2011). If this generalization is true, then turritellines would be expected to have most drill holes at site II (the most common vulnerable site during both crawling and sedentary life modes). Allmon et al. (1990) showed preference for abapertural quadrants in fossil turritellines, but we found no such preference for a particular quadrant (Fig. 5; Hagadorn & Boyajian, 1997). This suggests that there may be significant species-specific variation in this aspect of site selection, perhaps related to the prey’s escape behaviour (Kabat & Kohn, 1986), or that individuals of the same species may sometimes remain infaunal and sometimes crawl over the substrate (Waite & Strasser, 2011).

Correlation between predator size and prey size is another important criterion for evaluating size selectivity by predators. In *T. attenuata*, the overall correlation is poor (Fig. 3A), but the sample from Digha shows better results. This is mainly because naticid diversity and abundance are less in Digha, mostly dominated by *Polinices didyma* (Röding, 1798) and *Natica tigrina* (Röding, 1798), whereas in Chandipur naticid diversity and abundance are very high (Subba Rao et al., 1991) and adult size of the predators range from 1.4 cm (*Eunaticina* sp.) to 5.2 cm (*Polinices didyma*). It may be that mul-

tiple naticid species may attack *T. attenuata*, and such multispecies predation may blur the correlation between a specific predator and its prey. *Turritella duplicata* whereas shows relatively good correlation between outer borehole diameter (OBD) and prey size (Fig. 3B).

Anti-Drilling Prey Features

It appears that the less ornamented *T. attenuata* shows the maximum DF, whereas the more strongly sculptured *T. acutangula* has the lowest value. This pattern is true for both smaller and larger individuals (Table 1). For all 14 Indian species, a relation also exists between DF and the strength of the ornamentation. DF decreases with increasing ornamental index (Table 2) as well as size (Table 1).

Dudley & Vermeij (1978) demonstrated that ornamentation in turritelline species provides resistance to drilling. Strongly ornamented forms are less frequently preyed upon. Allmon et al., (1990) supported this general trend, but mentioned that this is not consistent when only the most ornamented forms are studied. Our results do not support the observation made by Allmon et al. (1990). For the turritellines of the Indian sub-continent, the species having relatively weaker ornamentation show higher drilling frequency while the stronger sculpture show low drilling intensity (Table 2). This trend holds good for all size classes. Out of 13 Indian species, eight of them (61.54%) belong to strongly ornamented classes (see Table 2). The biogeography of ornamented species of Indian turritellines shows a distinct pattern. The more strongly ornamented species (class 4), for example *T. acutangula*, live in the open marine southern coast of Indian peninsula. *Turritella attenuata*, which is more weakly ornamented, lives in restricted bays on the eastern coast (Fig. 1). The other, more strongly ornamented species are found mostly in the more highly agitated waters of the Andaman Islands (Subba Rao & Dey, 2000). It is a common observation that strength and the number of ribs in bivalve genera (e.g., *Anadara* and *Cardium*) increase in high energy environments (Stanton & Dodd, 1970; Alexander, 1974). Strong spiral ornamentation in turritellines may resist drilling predation, but is less effective against peeling predation. Axial ridges are better resistant to shell breakage (Vermeij, 1982). Spiral ribbing characterises the entire subfamily Turritellinae (Marwick & Hutt, 1957), and turritellines may therefore be phylogenetically constrained (cf., Seilacher, 1970), preventing them from evolving axial ornamentation.

Peeling Predation

Peeling Frequency

The average PF on Recent turritelline gastropods from Indian subcontinent is 1.62 ($n = 898$). This PF is comparable to that of all turritelline gastropods worldwide, which is 1.47 ($n = 1561$) ($p = 0.7701$). The general trend of peeling predation in turritelline gastropods for both the Indian subcontinent and the world is that predation frequency varies with prey size class. Smaller individuals, less than 4cm in length, show lower peeling frequency (0.5) compared to the larger size class (2.02) (see Table 1). Allmon et al. (1990) also made similar observations for fossil turritelline gastropods (Miller, 1975; Zipser & Vermeij, 1978; Vermeij, 1982; Vermeij & Dudley, 1982; but see Kabat & Kohn, 1986). Vermeij et al. (1980) reasoned that the larger shell is likely to have sustained more non-lethal attacks. The low PF in young specimens of larger species or adult specimens of smaller species may be due to the fact that they live predominantly infaunally (Waite & Strasser, 2011) and may thus escape the notice of epifaunal crabs (Campbell & Fielder, 1986).

Anti-Peeling Prey Features

In case of high spired forms, PF may vary with shell geometry (Zipser & Vermeij, 1978; Vermeij, 1981; Signor, 1985). In recent terebrid species, inflated forms show a higher incidence of repair scars than slender species (Signor, 1985). Allmon et al. (1990), however, did not find such a correlation in turritelline fossil assemblages since the Cretaceous, and our analysis is consistent with their results (Fig. 7). Both slender and inflated forms suffer a wide range of PF values. Within slender species there is no particular trend of decreasing PF with the increase in degree of inflation. Instead, a good correlation between ornamental strength and peeling frequency reveals specific prey adaptation against peeling (Vermeij, 1981). Highly ornamented forms (class 3 and 4) have a lower value of peeling frequency than weakly ribbed species (class 1 and 2, $p < 0.01$, chi-square test of independence: see also Fig. 7). Two species, however, show anomalous positions contrary to our expectations. The position of *T. bacillum* ($n = 2$; class 1) and *T. infraconstricta* ($n = 5$; class 4) may be due to low sample size.

Strongly ornamented shells might be expected to have lower values of peeling frequencies (Allmon, 1990). Perhaps the pattern in turritellines is due to the style of the ornamentation. Ornaments in terebrid gastropods are mainly

axial and the outer lip of the aperture is thick. These features resist shell breakage due to peeling. But in case of turritelline gastropods the outer lip is always thin and ornaments are spiral and these make the shell less effective against peeling predation. If any breakage occurs it will therefore penetrate further into the whorl than in terebrids. Paleozoic ammonoids may provide an analogous case. Many genera of ammonoids during Palaeozoic were spirally ribbed, but during the Mesozoic, the ribbing became mostly radial, which would perhaps align breakage parallel to the peristome margin and cause less damage to the whorl. For this reason, the evolution of radial ribs in ammonoids has been explained as the development of breakage-resistant features against the injuries mainly caused by decapod crustaceans (Ward & Wicksten, 1980; Ward, 1981).

Spatial Variability

Latitudinal variation in predation frequencies is evident for turritelline gastropods (Table 4). There is a steady decrease of DF, from 55.7% in low latitude (0° – 10°) areas to 6.7% in high latitude areas (60° – 70°), except the areas lying within the (50° – 60°) latitudinal bin, which records 0%. This may be due to the small number of specimens ($n = 28$) from this area. Previously, Dudley & Vermeij (1978) also estimated the latitudinal variation of DF and our expanded data supports their results.

The PF in Recent turritellines shows a broad latitudinal trend. Tropical, or near tropical (0° – 20°) species show higher incidences of PF (3.21 to 1.85) than their temporal (20° – 40°) counterparts (0.59 to 0.83), but PF again increases (1.58 to 2.83) in higher (40° to 60°) latitudinal bin (Table 4). This may be due to low sample size ($n = 20$). Similar latitudinal trends have been also observed in terebrid and other gastropods (e.g., thaidids and littorinids; Vermeij, 1982, and references therein).

CONCLUSIONS

(1) Drilling and peeling frequencies in Recent turritellines vary spatially; frequencies of predation decrease towards higher latitudes. Pooled frequencies of predation may therefore be of limited utility for testing specific evolutionary-ecological hypotheses, even in a taxon-level study.

(2) In general, smaller individuals show higher drilling and lower peeling frequencies. When all species are pooled together, this relation

becomes blurred for drilling predation. These patterns are consistent with the hypothesis that shell ornamentation is an anti-drilling trait in turritellines but less effective against peeling predation.

(3) Size-selectivity is widely variable both intraspecifically and interspecifically. For this reason, size selectivity may not be useful in testing hypotheses of escalation in this group.

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APPENDIX 1. New global dataset of drilling and peeling predation on Recent turritelline gastropods.

Family Turritellidae	n	No of drill holes	No of repear scar	DF (%)	PF	Authors
<i>T. attenuata</i>	815	97	1,973	12%	2.42	Present Data
<i>T. duplicata</i>	193	32	173	17%	0.9	Present Data
<i>T. acutangula</i>	65	3	56	5%	0.86	Present Data
<i>T. bacillum</i>	2	1	0	0%	0.5	Present Data
<i>T. terebra</i>	5	20	0	0%	4	Present Data
<i>T. columnaris</i>	47	10	65	21%	1.38	Present Data
<i>T. trisulcata</i>	39	8	20	21%	0.51	Present Data
<i>T. nebulosa</i>	1	1	0	100%	0	Present Data
<i>T. capensis</i>	20	2	7	10%	0.35	Present Data
<i>T. fascialis</i>	26	10	3	38%	0.16	Present Data
<i>T. vitullata</i>	17	1	14	6%	0.82	Present Data
<i>T. sinuata</i>	14	4	11	29%	0.79	Present Data
<i>T. carinifera</i>	17	1	26	6%	1.53	Present Data
<i>T. nivea</i>	30	3	7	10%	0.23	Present Data
<i>T. fastigiata</i>	9	2	7	22%	0.78	Present Data
<i>T. infraconstricta</i>	6	0	5	0%	0.83	Present Data
<i>T. monilifera</i>	1	0	3	0%	3	Present Data
<i>T. rosea</i>	12	0	19	0%	1.58	Present Data
<i>T. variegatus</i>	5	0	18	0%	3.6	Present Data
<i>T. fuscocincta</i>	31	0	8	0%	0.26	Present Data
<i>T. hanleyana</i>	1	0	9	0%	9	Present Data
<i>T. cornea</i>	8	0	22	0%	2.75	Present Data
<i>T. exolata</i>	18	0	9	0%	0.5	Present Data
<i>T. maculata</i>	8	3	13	38%	1.63	Present Data
<i>T. erosa</i>	28	4	-	14.3%	-	Dudley & Vermeij, 1978
<i>T. erosa</i>	70	6	-	8.6%	-	Dudley & Vermeij, 1978
<i>T. erosa</i>	11	0	-	0%	-	Dudley & Vermeij, 1978
<i>T. erosa</i>	48	5	-	10.4%	-	Dudley & Vermeij, 1978
<i>T. reticulata</i>	29	0	-	0%	-	Dudley & Vermeij, 1978
<i>T. reticulata</i>	27	1	-	3.7%	-	Dudley & Vermeij, 1978
<i>T. communis</i>	26	7	-	27.7%	-	Dudley & Vermeij, 1978
<i>T. communis</i>	30	1	-	3.3%	-	Dudley & Vermeij, 1978
<i>T. communis</i>	9	0	-	0%	-	Dudley & Vermeij, 1978
<i>T. variegata</i>	11	0	-	0%	-	Dudley & Vermeij, 1978
<i>T. nodulosa</i>	116	32	-	27.6%	-	Dudley & Vermeij, 1978
<i>T. acropora</i>	13	2	-	15.4%	-	Dudley & Vermeij, 1978
<i>T. acropora</i>	22	0	-	0%	-	Dudley & Vermeij, 1978
<i>T. terebra</i>	33	5	-	15.2%	-	Dudley & Vermeij, 1978
<i>T. terebra</i>	18	5	-	27.8%	-	Dudley & Vermeij, 1978

(continues)

(continued)

Family Turritellidae	n	No of drill holes	No of repear scar	DF (%)	PF	Authors
<i>T. terebra</i>	12	2	-	16.7%	-	Dudley & Vermeij, 1978
<i>T. duplicata</i>	39	2	-	5.2%	-	Dudley & Vermeij, 1978
<i>T. sp.</i>	27	10	-	37%	-	Dudley & Vermeij, 1978
<i>T. leucostom</i>	156	80	-	51.3%	-	Tull & Bohning-Gaese, 1993
<i>T. gonostom</i>	172	5	-	2.9%	-	Tull & Bohning-Gaese, 1993
<i>T. acropora</i>	6	1	-	12%	-	Dudley & Vermeij, 1978
<i>T. annulata</i>	436	173	-	62.6%	-	Buchanan, 1958
<i>T. banksi</i>	12	2	-	16.7%	-	Dudley &Vermeij, 1978
<i>T. banksi</i>	9	0	-	0%	-	Dudley &Vermeij, 1978
<i>T. carinifera</i>	13	0	-	0%	-	Dudley &Vermeij, 1978
<i>T. communis</i>	15	3	-	20%	-	Dudley &Vermeij, 1978
<i>T. exoleta</i>	48	2	-	4.3%	-	Dudley & Vermeij, 1978
<i>T. gonostoma</i>	70	28	-	40%	-	Dudley & Vermeij, 1978
<i>T. leucostoma</i>	35	9	-	26%	-	Dudley & Vermeij, 1978
<i>T. mariana</i>	36	13	-	36%	-	Dudley & Vermeij, 1978
<i>T. nodulosa</i>	35	8	-	22.9%	-	Dudley & Vermeij, 1978
<i>T. pagoda</i>	50	1	-	2%	-	Dudley & Vermeij, 1978
<i>T. symmetrica</i>	57	11	-	19.3%	-	Dudley & Vermeij, 1978
<i>T. triplicata</i>	34	16	-	47.1%	-	Dudley & Vermeij, 1978
<i>T. variegata</i>	42	15	-	35.7%	-	Dudley & Vermeij, 1978
<i>T. sp.</i>	47	9	-	19.1%	-	Dudley & Vermeij, 1978
<i>T. sp.</i>	138	94	-	68.1%	-	Dudley & Vermeij, 1978
<i>T. sp.</i>	61	6	-	9.8%	-	Dudley & Vermeij, 1978
<i>T. communis</i>	2,209	973	-	44%	-	Sawyer & Zuchin, 2010
<i>T. gonostoma</i>	107	-	94	-	0.88	Gerhard et al., 1997
Total	5,640	1,729	2,562	31%	1.71	

LIFE HISTORY PARAMETERS IN THE EDIBLE SNAIL *ADELOMELON ANCILLA* FROM PATAGONIAN WATERS

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Martín I. Brogger² & Gregorio Bigatti¹

ABSTRACT

Life history parameters, including size-age, production, mortality and size at reproductive maturity, were studied on the edible snail *Adelomelon ancilla* to provide useful management data. Stable oxygen isotope ratios confirmed semiannual formation of internal growth marks. *Adelomelon ancilla* can reach up to 18 years at a size of 150 mm, fitting to a Gompertz growth model. Size at which 50% of the population matured was 73.5 mm in males (7 years) and 93.5 mm in females (9 years). The long lifespan of this species is comparable to other large gastropods, in particular volutids from southern Atlantic Ocean. Longevity, low somatic production, low population density, late reproductive maturity and direct development with few embryos, makes *A. ancilla* a vulnerable resource that will require a proper management approach incorporating estimates of these life history parameters.

Key words: age, growth, fisheries management, conservation, oxygen stable isotopes.

INTRODUCTION

The growth rate of an organism provides basic ecological data and is one of the prime parameters to describe population dynamics. Furthermore, fisheries managed with size limits require accurate estimates of age and growth to protect reproductive capacity of stocks and to estimate the sustainable stock yield (Prince & Shepherd, 1992; Jennings et al., 2001; Hilborn & Walters, 2004; King, 2007). In terms of tonnage and economic value, marine mollusks are among the most important invertebrate fishery resources in the world (FAO, 1998a, b). Marine gastropods represent about 2% of world mollusk catches (Leiva & Castilla, 2002). Fishing statistics show an increase in the number of countries in the gastropod world landing records, rising from 23 in 1979 to 47 in 2010 (FAO, 2010). Catches of mollusks have decreased significantly since 2002, and this may be due to their extreme vulnerability to habitat degradation, overexploitation, and predation by alien species (FAO, 2010). More than 50% of marine resources are overexploited, and food demands are increasing with the growing population (Lodeiros, 2011). Currently, a

scallop fishery is considered the only mollusk fishery of significance for the economy in northern Patagonia (Ciocco et al., 2006). However, gastropods of the family Volutidae have been identified as a potential resource for artisanal diving fisheries, although to date most catches are sporadic and small in the north Patagonic gulfs (Ciocco, 1995, 1999; Bigatti & Ciocco, 2008, Bigatti et al., 2008), but the interest in gastropod fisheries is increasing in the zone. In the case of volutid gastropods, large body size and somatic production, coupled with slow growth rate, late reproductive maturity and direct development make this group extremely vulnerable to overexploitation (Bigatti et al., 2007). For this reason, typical body sizes at specific ages, size/age at reproductive maturity and growth estimates are necessary for effective fisheries management of the species. The marine snail *Adelomelon ancilla* (Lightfoot, 1786) is a neogastropod belonging to the family Volutidae. This species is distributed along the southern tip of South America, from 30°S (Brazil) in the southwestern Atlantic to Ushuaia in the Beagle Channel and northward into the Pacific to Isla Chiloé at 25°S in central Chile (Castellanos & Landoni, 1992; Zabala,

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2013). *Adelomelon ancilla* is dioecious, with internal fertilization. Females attached the egg-capsules to hard substrata. Hatching occurs at crawling stage, after complete intracapsular development, feeding on the intracapsular fluid (Penchaszadeh et al., 1999, Penchaszadeh et al., 2009). This species is a top-predator of benthic communities, preying principally on other gastropods and on bivalves (Botto et al., 2006; Bigatti et al., 2009; Zabala et al., 2013).

Tag-recapture techniques, analyses of modal shift in sizes of cohorts, and studies of growth layers in the shell spire, statoliths or opercula are commonly used to estimate growth rates of gastropods (Tarr, 1992; Richardson et al., 2005; Henry & Jarne, 2007). However, the effects of tagging (McShane, 1988) and uncertainty in the rate of production of growth layers observed (Day & Fleming, 1992; McShane & Smith, 1992; Day et al., 1995) have meant that estimates of age and growth cannot be made with precision using these techniques. The use of oxygen stable isotopes (OSI) analysis is proposed as an alternative method for aging shells of gastropods and determining growth rates (Gurney et al., 2005). Epstein et al. (1951, 1953) established a relationship between seawater temperature and the relative abundance of oxygen stable isotopes in the calcium carbonate of the shells of mollusks and other marine invertebrates. Subsequently, many studies have used OSI to determine growth rates and age for various gastropods (e.g., Panarello, 1987; Geary et al., 1992; Cespuglio et al., 1999; Kennedy et al., 2001; Keller et al., 2002; Cledón et al., 2005a; Giménez et al., 2004; Bigatti et al., 2007; Arrighetti et al., 2011, 2012). The application of this technique is possible when the shell is laid down in isotopic equilibrium with the seawater, the salinity is known or constant, and seasonal fluctuation in seawater temperature is such that the magnitude of the corresponding seasonal oscillations in OSI values are discernible. In the study site, water temperature ranges between 9.7°C (late winter) to 18.3°C (late summer) and salinity is rather constant at 34‰ (Rivas & Beier, 1990), so these oscillations permit determine age and growth rate (Gurney et al., 2005) of *A. ancilla*.

The main goal of this work was to study life history parameters of *A. ancilla* in Golfo Nuevo, Patagonia, particularly examining its growth, age and size at reproductive maturity, mortality and production, which will generate data useful for future management of this valuable resource.

MATERIALS AND METHODS

Sampling and Morphometric Parameters

Specimens of *Adelomelon ancilla* were captured monthly during 3 years (2001–2004), in Golfo Nuevo, Patagonia (42°48'S, 64°54'W) by SCUBA diving between 5 to 15 m depth (according to the tide). In order to test the population density, sampling was performed using three random transects per month crossing 100 m in length and 2 m wide, parallel to the coast, at the same depth. Animals were taken to the laboratory, where soft parts were removed from the shell of live animals using a press. Shell length (SL) and male penis length (PL) were measured with a vernier caliper and spiral growth trajectory (SG) was also measured with a thread and caliper to the nearest millimeter (Fig. 2). Shell-free wet mass (SFWM) was determined to the nearest gram after removing marine water from the tissues using a paper towel. The relationships between these morphometric parameters were described by linear regression. Each individual was sexed by the presence or absence (female or male, respectively) of reproductive accessory glands (albumen and capsule gland) and vagina. The sex ratio was compared to parity (1:1) with the χ^2 test.

Age and Growth

In order to confirm if external shell growth marks (Fig. 1) corresponded to internal growth band pattern, the shells of 11 individuals were cut along the whorls following the spiral growth trajectory from the apex to the posterior end of the aperture (Fig. 2). This set of specimens was collected at the same time. The cut surface was ground with sandpaper, using grits of P400, P1200, P2400 and P4000 grade and subsequently polished using a 1 μ m polycrystalline diamond suspension. The polished shell cuts were examined by stereomicroscope. Visible internal growth marks were identified (Fig. 4) on both sides of the shell, and the corresponding growth trajectory length SG_i from the apex to the mark *i* was measured. X-rays photographs of the shells (Fig. 3) were taken to confirm internal growth marks with an X (RX) LADEM/VETTER Rem-150 instrument and the follow parameters settings: 50 mA, 30 KV voltage and 0.06 s exposure time.

Periodicity of growth band formation was evaluated by means of oxygen stable isotope (OSI) analysis ($\delta^{18}\text{O}$, ratio of isotopes $^{18}\text{O}/^{16}\text{O}$). $\delta^{18}\text{O}$ in biogenic calcium carbonate structures

is negatively correlated with temperature during shell deposition. This relation is nearly linear between 5°C and 30°C, as demonstrated empirically by Epstein & Lowenstam (1953) and Epstein et al. (1953). Three representative specimens, two females of 130 mm (Fig. 8) and 150 mm (Fig. 6), and one male of 138 mm SL (Fig. 7) were used for OSI analysis. We extracted carbonate samples (approximately 10 mg each) from the shell prismatic layer along the SG by means of a dremel (bit size 0.5 mm) at a sample-to-sample distance of 1 to 3 mm, when possible (70 samples from individual in Fig. 6, 65 from individual in Fig. 7 and 45 samples from individual in Fig. 8). The first sample (SG ~ 0 mm) was taken near the apex; meanwhile the last samples were taken closer to the apertural lip. Oxygen isotope analyses were carried out in the INGEIS-UBA laboratories following the usual techniques (McCrea, 1950; Sharp, 2007). Samples were reacted with “100%” H_3PO_4 , cryogenically purified and measured in a Finnigan MAT Delta S, triple collector, double inlet system, mass spectrometer, against the working standard, “carrara marble”. All data are reported in the conventional per mil deviation ($\delta^{18}\text{O}\text{‰}$), defined as:

$$\delta^{18}\text{O} (\text{‰}) = \left[\frac{(^{18}\text{O}/^{16}\text{O})_S - (^{18}\text{O}/^{16}\text{O})_R}{(^{18}\text{O}/^{16}\text{O})_R} \right] \times 1000$$

and referred to the V-PDB standard, through the NBS-19 (NIST) reference material (Gonfiantini, 1978, 1984). The analytical uncertainty is $\pm 0.1\text{‰}$ (2σ based on repeated analysis of a laboratory working standard over a 30-year period). $\delta^{18}\text{O}$ values were converted into water temperature by the paleotemperature equation of Epstein et al. (1953), as modified by Craig (1965) and Anderson & Arthur (1983):

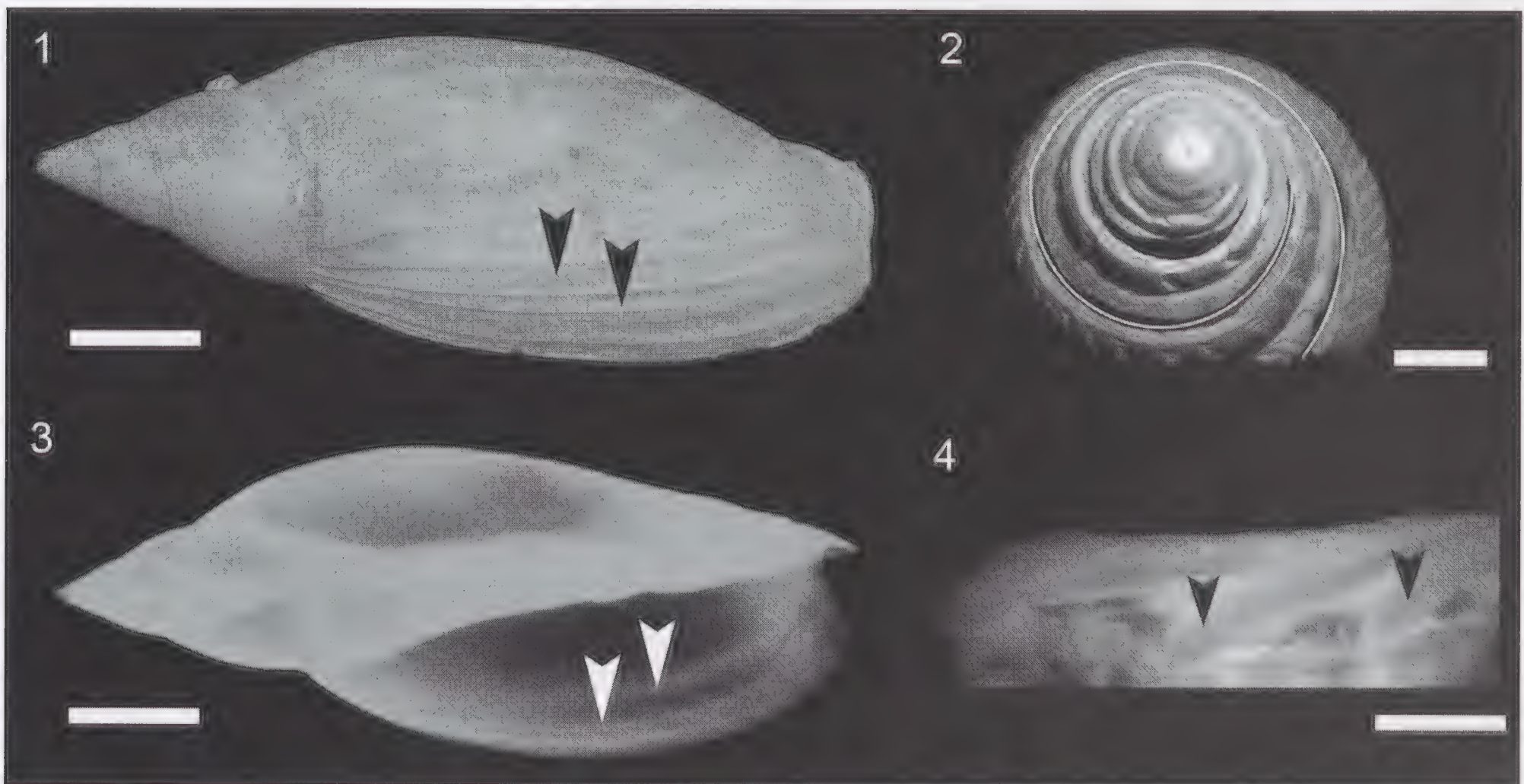
$$T(^{\circ}\text{C}) = 20.6 - 4.34 (\delta^{18}\text{O}_{\text{aragonite}} - \delta^{18}\text{O}_{\text{water}} + 0.27\text{‰})$$

Sea water $\delta^{18}\text{O}_{\text{water}}$ was estimated at -0.30‰ from the gridded data (version 1.1) of Le Grande & Schmidt (2006, see also the corresponding NASA website <http://data.giss.nasa.gov/o18data>).

Visual shell marks coinciding with $\delta^{18}\text{O}$ peaks were interpreted as late winter (maximum) or late summer (minimum) growth marks, and the distance between two subsequent late winter or late summer growth marks as the annual shell increment. Thus, we interpreted each growth mark, and its corresponding shell growth trajectory length SG_i , as a size-at-age data pair. SG_i was translated into shell length SL_i by means of the linear relationship between the two parameters. The iterative non-linear Newton algorithm was used to fit a growth model to the size-at-age data. The Gompertz growth model was found to fit these data best:

$$\text{SL}_t = \text{SL}_{\infty} * e^{-e[-K * (t - t_0)]}$$

where SL_{∞} is asymptotic shell length, K is the



FIGS. 1–4. *Adelomelon ancilla*. FIG. 1: External growth marks (arrows); FIG. 2: Spiral growth trajectory (SG), where the samples for oxygen isotopes were taken; FIG. 3: Internal growth marks (arrows) viewed by X-rays; FIG. 4: Detail of internal growth marks (arrows) under stereoscopic microscope. Scales bars Figs. 1–3 = 2 cm; Fig. 4 = 2 mm.

body growth coefficient, t is age and t_0 is the theoretical age at which length equals zero (Brey, 2001).

Overall growth performance was estimated by the index:

$$P = \log (K * SL_{\infty}^3)$$

and displayed in the form of an auximetric grid of $\log(K)$ versus $\log (SL_{\infty}^3)$, as described by Moreau et al. (1986) and Pauly et al. (1996).

Biomass and Production

Individual somatic production was calculated by the mass-specific growth rate P_i (SFWM) method according to Brey (1999, 2001) from the size-frequency distribution, the size-body function and the growth model parameters:

$$P_i = M_i * G_i$$

where M_i represents mean individual body mass at size i and G_i the annual mass specific growth rate at size S_i given by:

$$G_i = b * K * \ln (SL_{\infty} / SL_i)$$

where b represents the slope of the regression growth function.

Smaller sizes classes are likely to be under-sampled due to small individuals are very cryptic, living buried in the substratum. In addition, gastropods collection was performed by SCUBA diving. Thus, population production (P) and production-to-biomass ratio (P/B) based on size distribution were not calculated.

Mortality

Mortality is one of the processes regulating size and structure of a population. There are different types of mortality; here we use mortality as “the monotonous decrease in number of an age class with time, a process caused by predation, parasitism and diseases” (Brey, 2001). We presumed that mortality in *A. ancilla* can be described by the single negative exponential mortality model based on a virtual handbook (Brey, 2001) and used the corresponding size-converted catch curve (Pauly, 1984a, b) to estimate total mortality rate Z (y^{-1}):

$$N_j / \Delta t_j = N_0 * e^{-Z t_j}$$

where N_j is the number in the size class j , Δt_j is time required to grow through this size class j , and t_j is age at midsize of size class j . Size-frequency data (SFD) by themselves are not sufficient for an analysis of mortality. Because mortality is defined as decrease in numbers with time and SFD show changes in numbers with size, we have to “translate” size into time (= age). This can be achieved by a growth function. Hence, to derive information on mortality from SFD, we need both a size-frequency distribution and a growth function. This curve was based on the size-frequency distribution of the individuals sampled and the Gompertz growth function (Brey 1999; 2001).

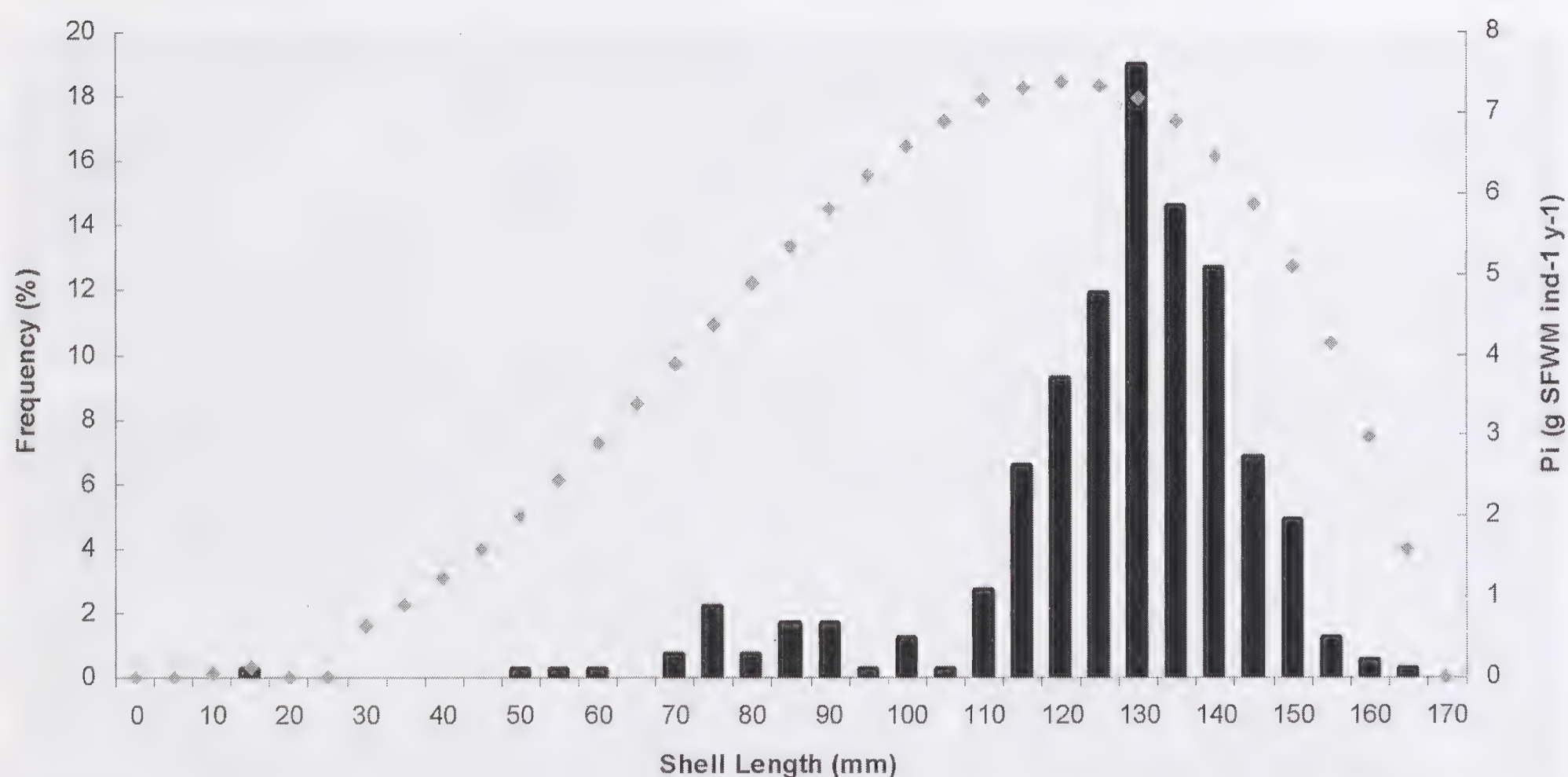
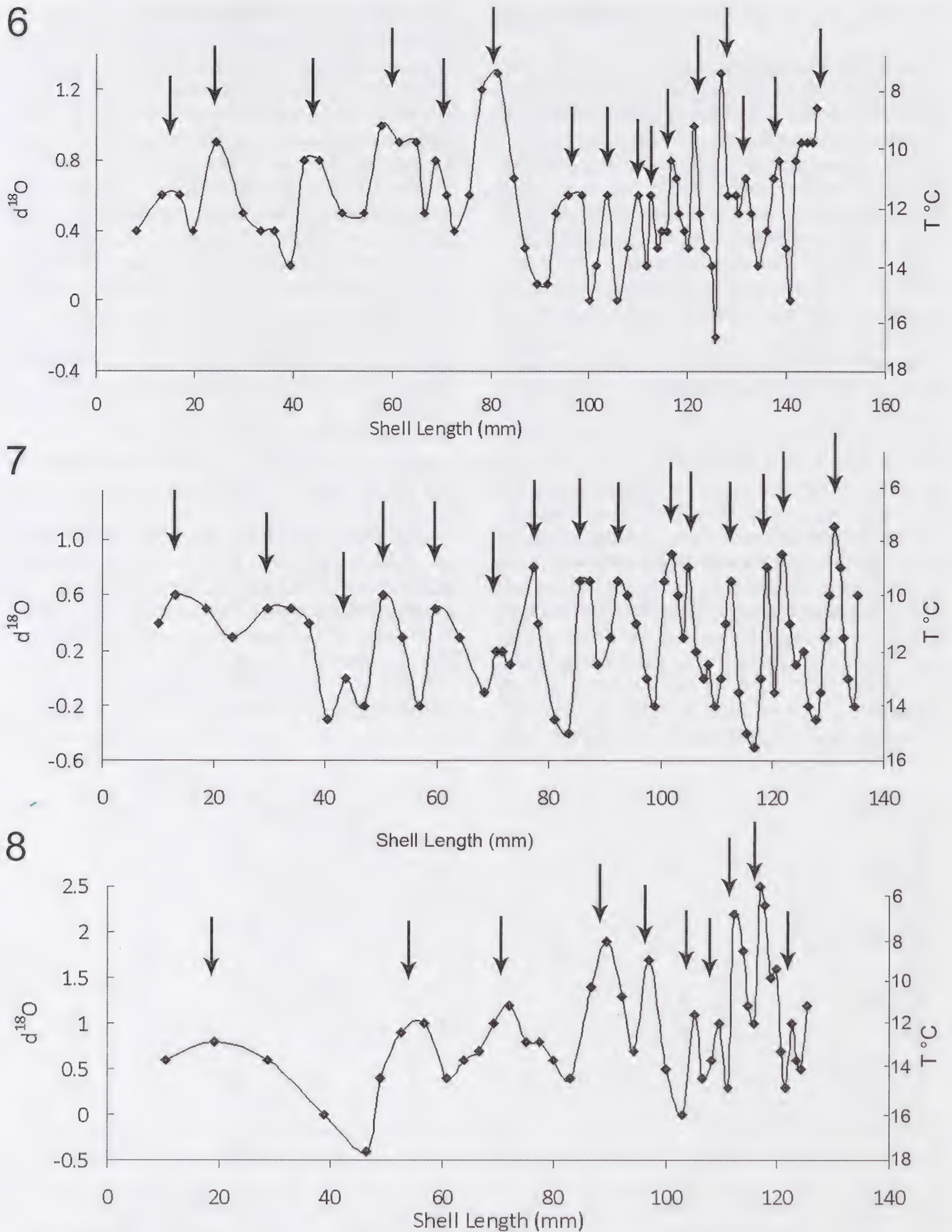


FIG. 5. Size-frequency distribution of *Adelomelon ancilla* ($N = 234$) from Golfo Nuevo area. Superimposed dotted line indicates individual somatic production P_i (g SFWM year $^{-1}$) per size class.



FIGS. 6–8. $\delta^{18}\text{O}$ profile corresponding to the shell length of three individuals of *Adelomelon ancilla*. FIG. 6: 150 mm SL; FIG. 7: 138 mm SL; FIG. 8: 130 mm SL. Arrows indicate position of annual internal growth marks (maximum) formed at low water temperatures (late winter). Water temperature (right hand scale) computed from carbonate $\delta^{18}\text{O}$ by the paleotemperature equation of Epstein et al. (1953), see text for details.

Size, Weight and Age at Reproductive Maturity

Samples of gonad of both sexes were processed by standard histology procedures in order to determinate the maturation stage. The detailed methods, gonad general structure and gametogenic cycle are described in Penchaszadeh et al. (2009). The individual size range used was 46 mm to 168 mm in shell length (SL). Individuals without gonad were assigned to the immature condition. The SL at gonadic maturation was determined as the minimum SL at which gonads were developed, for both sexes. Females and males were considered mature whenever their gonads presented vitellogenic oocytes or spermatozoa in the lumen of the tubules, respectively. Immature (stage = 0) females were characterized by the presence of undeveloped ovaries, lacking oocytes and oogenic stages or with early oocytes in pre-vitellogenesis process. Mature (stage = 1) females had developing ovaries, with growing and vitellogenic oocytes. Immature males were characterized by the absence of

developing or mature spermatozoa. Gonadic maturity was determined by whether stages of spermatogenesis, including the presence of mature spermatozoa into spermatogenic tubules. The mature-immature data set was plotted against SL to allow extrapolation of the minimum SL at maturation, and the SL at which 50% and 100% of the population was mature. To estimate size at which 50% of the population is sexually mature (SL_{50}), we calculated the SL at which half of the individuals sampled exhibited mature gonads (Gendron, 1992; Bigatti et al., 2008; Averbuj et al., 2010). To do so, logistic regressions of SL–gonadic maturity were applied to fit mature-immature data on an SL axis (mature = 0; immature = 1). The functions calculated were:

$Y = 1/(1+e^{-(a+bX)})$ and $SL_{50} = -a/b$ where Y represents gonadic maturation, X the shell length in mm (SL) and a , b were constants.

In addition, shell-free wet body mass (SFWM) vs. shell length (SL) of both sexes and male penis length (PL) vs. SL were calculated as additional maturation index (Gendron, 1992).

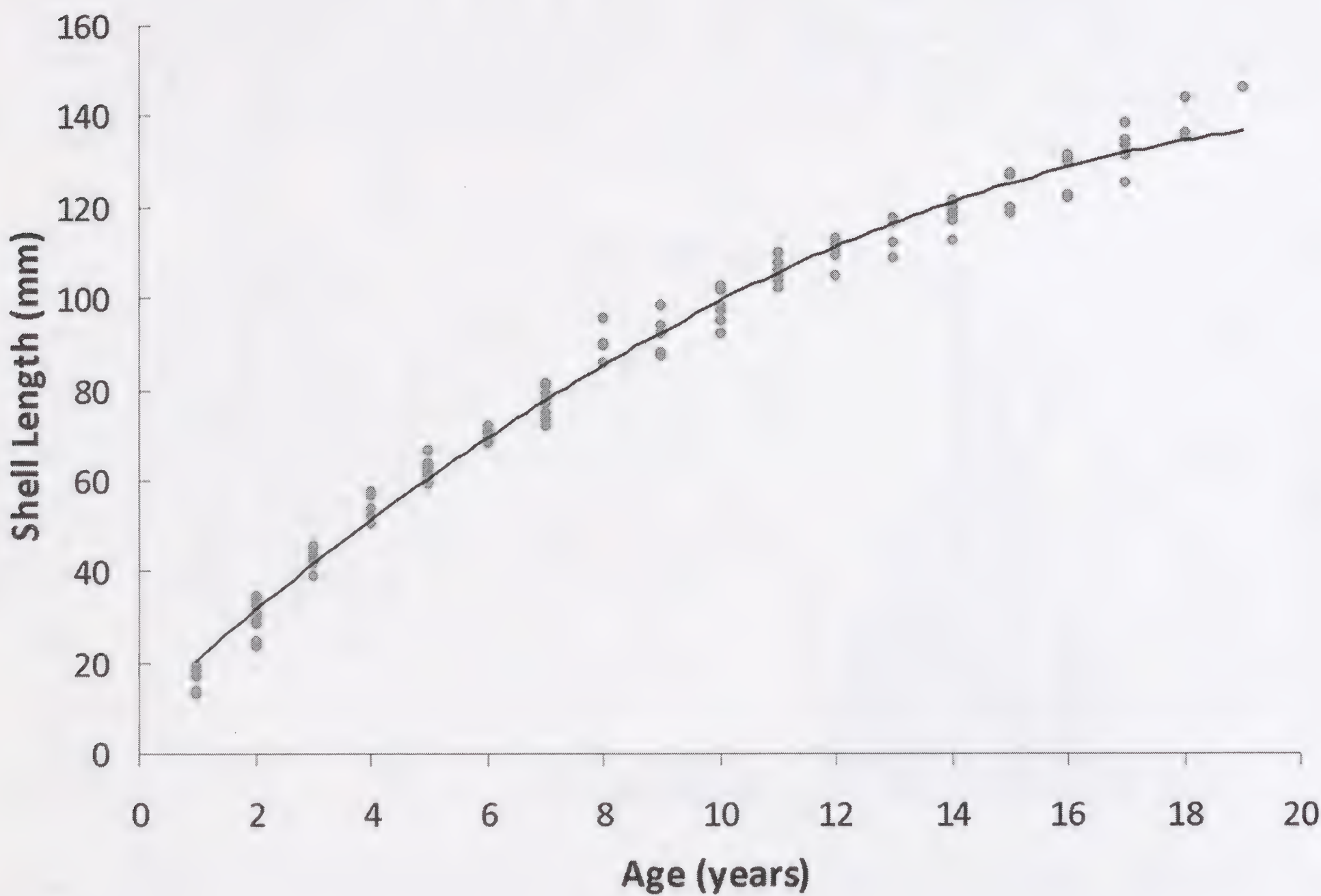


FIG. 9. Gompertz growth model for *A. ancilla*. $SL_t = 170 e^{-e(-0.140 * (t - 5.500))}$; $r^2 = 0.974$; $N = 142$ size-at-age data pairs obtained from the 11 specimens.

RESULTS

Age and Growth

A total of 234 individuals of *Adelomelon ancilla* were captured, ranging from 15 to 168 mm in total shell length (SL) (Fig. 5). Juveniles lesser than 15 mm were not registered, because they are very cryptic. Males showed an average of 128.0 ± 18.1 mm SL, while females showed an average of 130.3 ± 16.4 mm SL. The sex ratio of this population of *A. ancilla* (57% females and 43% males) was not statistically different from parity ($\chi^2 = 0.0196$, $df = 1$, $p = 0.888$) throughout the sampled period. Shell-free wet body mass ranged from 3 g to 139 g. Males showed an average of 70.4 ± 27.2 g of SFWM, while females showed an average of 67.4 ± 20.4 g of SFWM. SFWM was related to SL by:

$$\text{Log (SFWM)} = -4.3325 + 2.9082 \cdot \log \text{ SL};$$

$$(r^2 = 0.75; N = 234)$$

Growth trajectory length SG, that is, the distance from apex to measurement points along the growth spiral (Fig. 2) was linearly related to SL:

$$\text{SG} = 0.985 \text{ SL} + 52.600; (r^2 = 0.78; N = 175)$$

The density of *A. ancilla* from Golfo Nuevo, northern Patagonia, population ranged from 0.007 to 0.037 individual/m² (mean = 0.022 ± 0.021 individual/m²). The distribution of this population was observed in small groups within the transects.

The $\delta^{18}\text{O}$ profiles showed a distinct oscillating pattern in the analyzed shells (Figs. 6–8). The average range of -0.42‰ to $+1.63\text{‰}$ $\delta^{18}\text{O}$ corresponds to a temperature range of about 10.2°C to 17.5°C . The distinct grey colored shell growth marks visible in the shell cuts (Fig. 4) were situated close to the maximum and minimum $\delta^{18}\text{O}$ values (Figs. 6–8). Thus, one mark was formed at the end of the summer (minimum peak), and another at the end of the following winter (maximum peak). The internal marks were confirmed by X-rays (Fig. 3). The largest animal studied (Fig. 6), which measured 150 mm SL, revealed peaks corresponding to an age of 18 years.

The 142 size-at-age data pairs obtained from the 11 analyzed specimens best fitted to the Gompertz growth model (Fig. 9):

$$\text{SL}_t = 170 e^{-e^{(-0.140 \cdot (t - 5.500))}}; (r^2 = 0.974; N = 142)$$

Overall growth performance of *A. ancilla* was $P = 5.84$ (Fig. 10). The observed relationship between length and calculated SFWM of *A. ancilla* ($\text{Log (SFWM)} = -a + b \cdot \log \text{ SL}$) was used for production estimates. Individual somatic production (P_i) increased steadily with size to a maximum of $7.38 \text{ g SFWM year}^{-1}$ at 120 mm SL and decreased again thereafter. The distribution of total annual production P

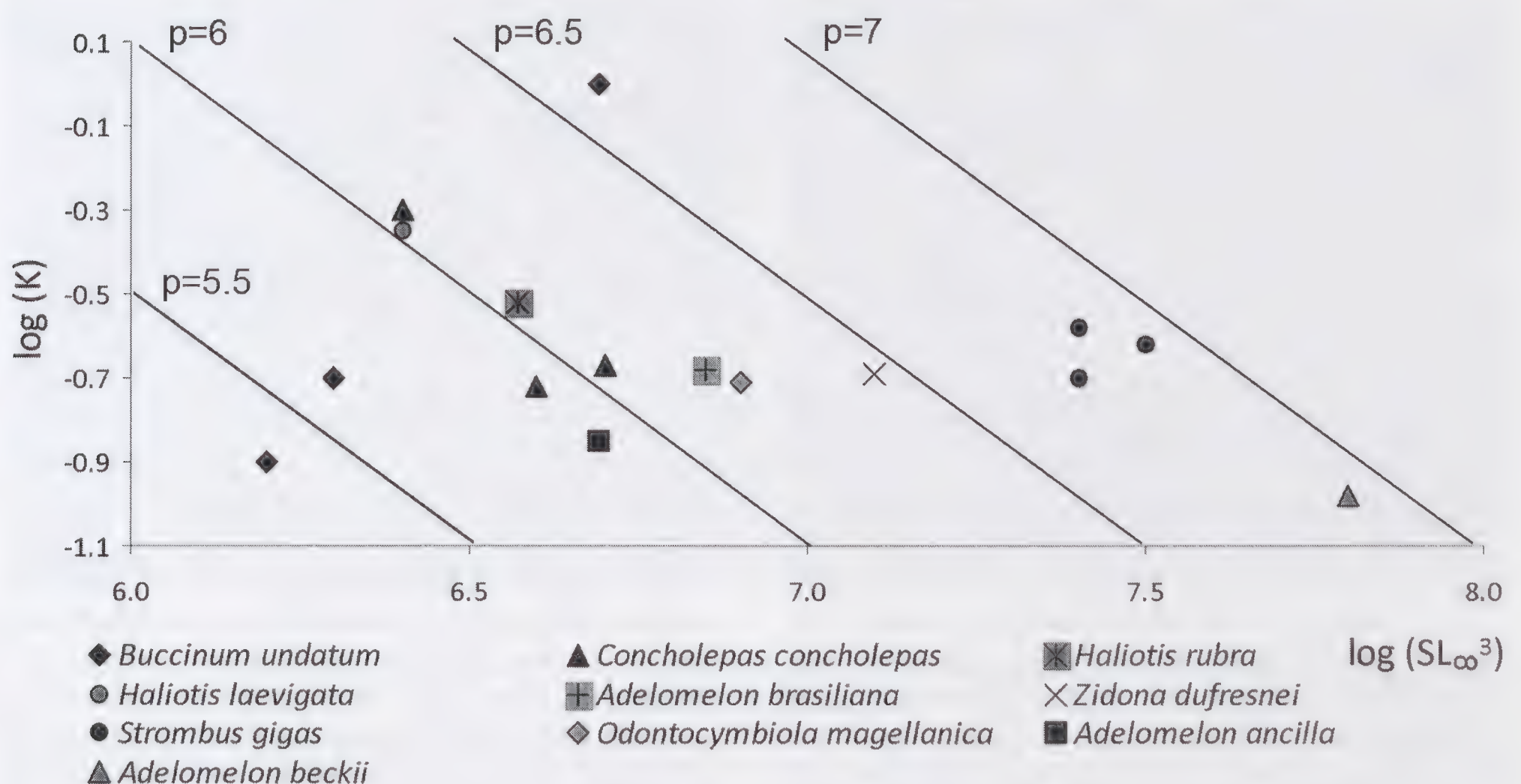


FIG. 10. Auximetric grid comparing growth performance in actual or potential marine gastropod resources. Growth performance index $P = \log (K \cdot \text{SL}_\infty^3)$.

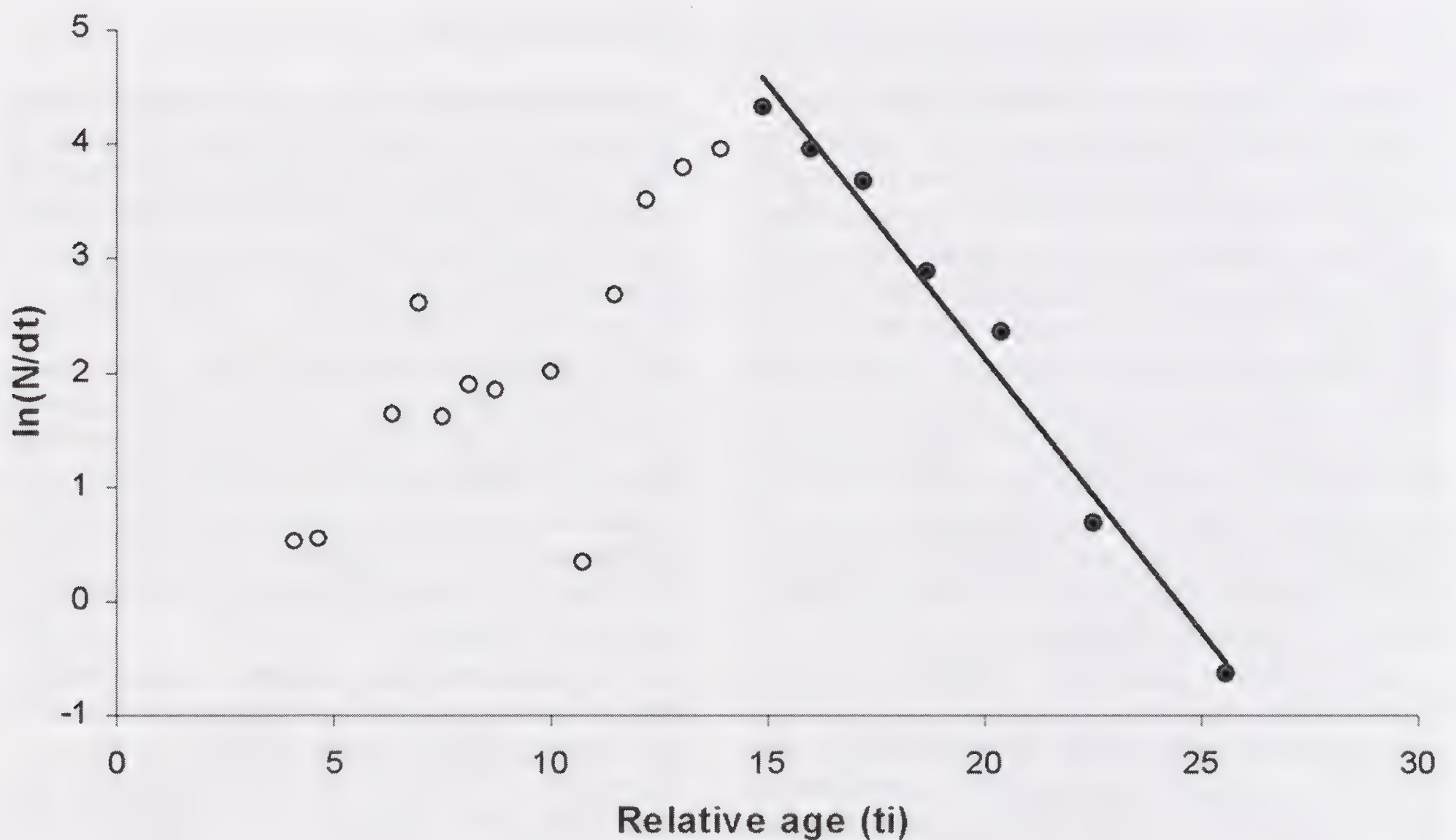


FIG. 11. Size-converted catch curve based on the size–frequency distribution (Fig. 5) and the growth function (Fig. 9) of *A. ancilla*. Dots: data included in regression; circles: data excluded from regression. $\ln(N/dt) = -0.436 ti + 10.941$; $r^2 = 0.98$; 95% confidence range of slope = ± 0.110 .

and the abundance among the size class are illustrated in Figure 5.

The size converted catch curve (Fig. 11) indicates that animals younger than 12 years are strongly under-represented in our sample. Total mortality rate Z of *A. ancilla* amounted to 0.063 y^{-1} (95% confidence range ± 0.110 , Fig. 11). Size-converted catch curve (Fig. 11) does not show the typical pattern of populations exploited by fisheries.

Size, Weight and Age at Reproductive Maturity

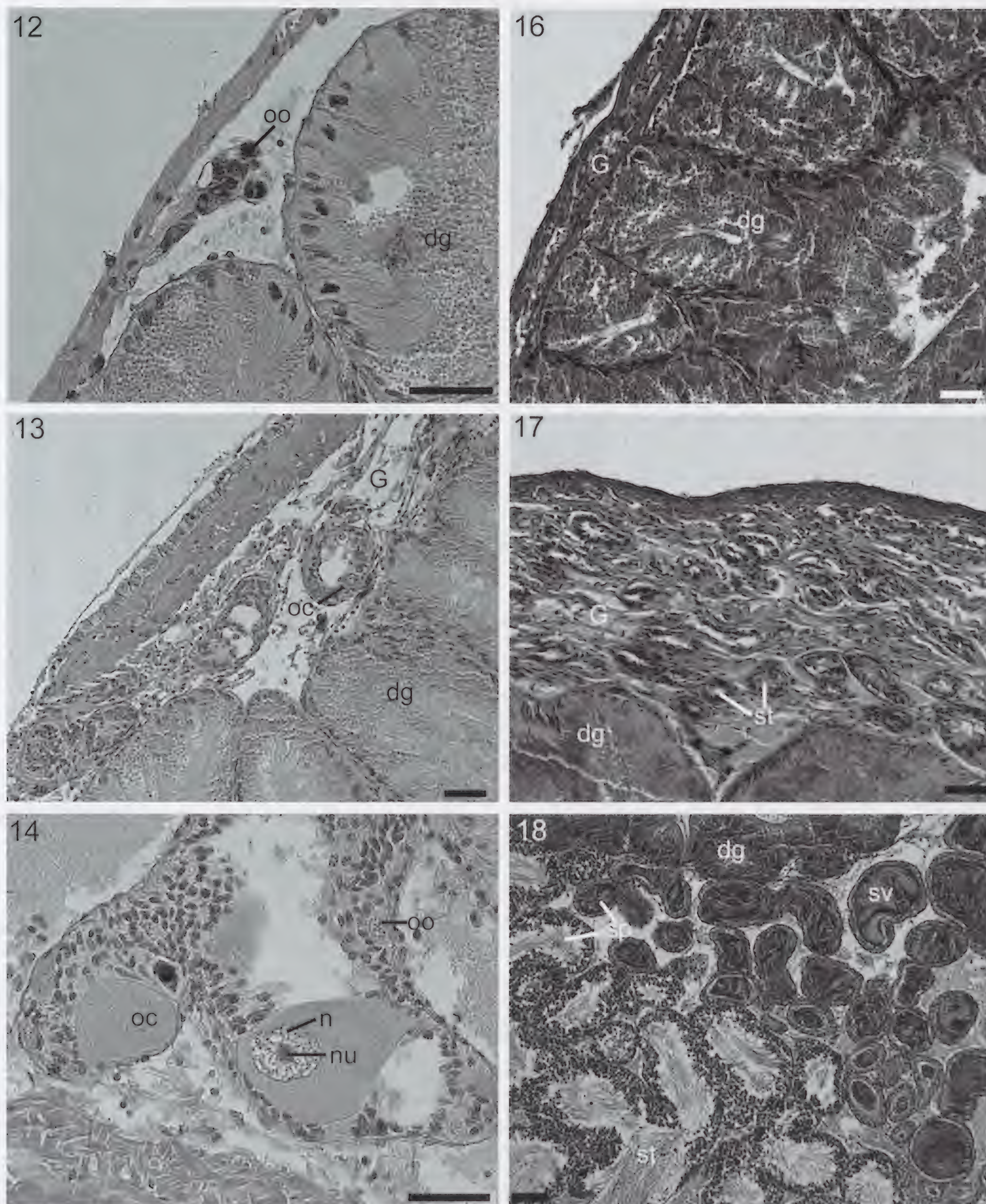
The histological examination showed immature and mature stages in both sexes, which were characterized in Figures 12–17. Females have vitellogenic oocytes at a minimum size of 93 mm in SL. The size at which all the females were mature was 97 mm (Fig. 20). Thus, the SL at which 50% of the female population was considered to be mature (SL_{50}) was 93.5 mm (Fig. 18). Males with mature spermatozoa (ready for evacuation) in the lumen of their tubules occurred at a minimum size of 67.5 mm in SL. At 88 mm in SL, all the males were mature (Fig. 21). The size at which 50% of the male population was considered to be mature (SL_{50}) was 73.5 mm (Fig. 19).

The SFWM inflexion point was similar in both sexes, 85 mm for females ($r^2 = 0.9033$; $N = 122$) (Fig. 22) and 86 mm for males ($r^2 = 0.9176$; $N = 112$) (Fig. 23). In both cases, the SFWM inflexion point in shell length was not coincident with the gonadic maturation size. On the other hand, a positive correlation between shell length and penis length in males was observed ($r^2 = 0.6514$; $N = 89$; $p < 0.05$). The penis structure in *A. ancilla* males developed prior to gonad maturation. Moreover, all males over 60 mm in SL had a conspicuous penis.

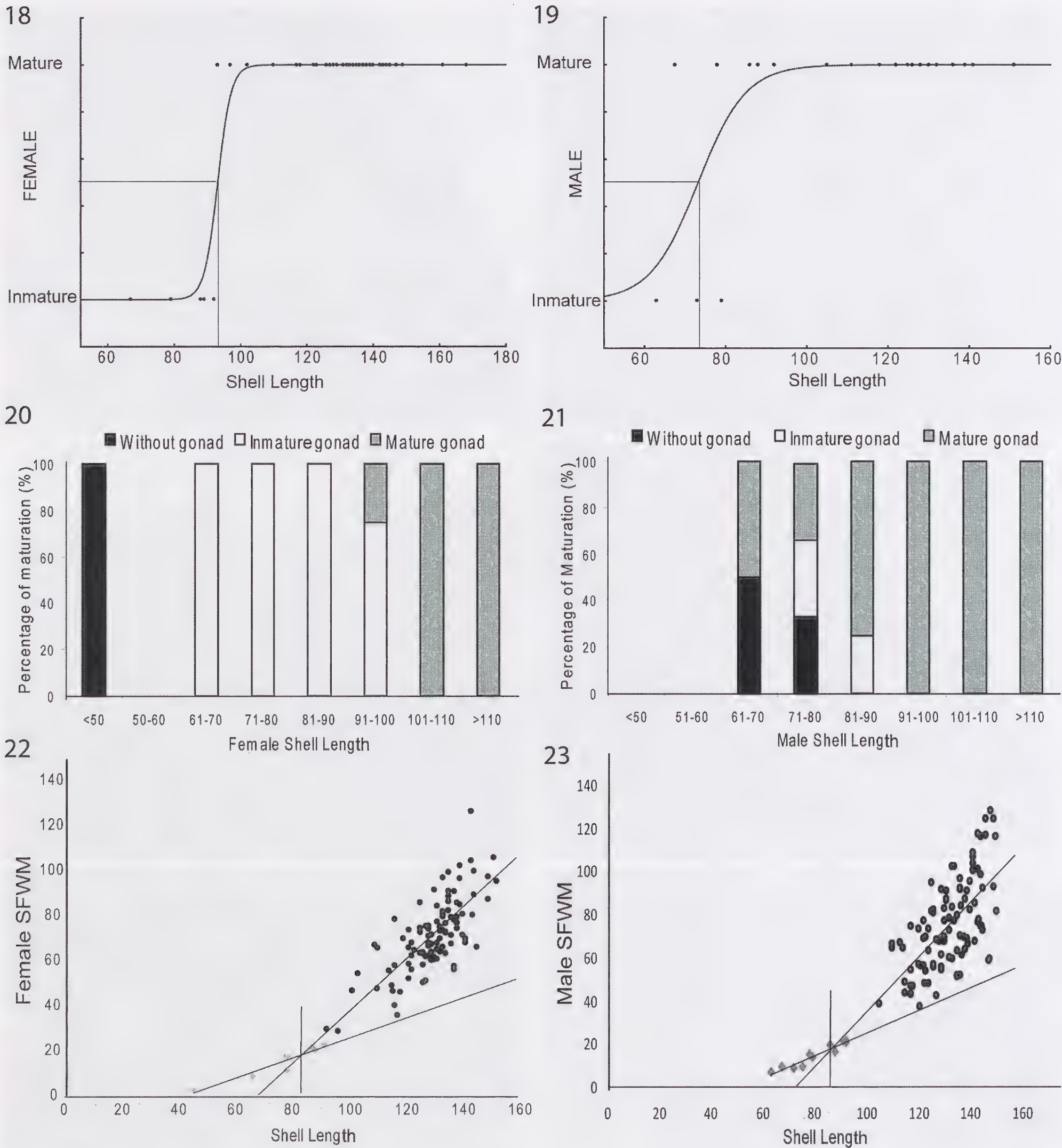
DISCUSSION

Stable Oxygen Ratios

The oxygen stable isotope (OSI) technique has the advantage of assessing age and growth related to the shell length in gastropods. OSI ratios may also be a useful tool to validate the frequency at which shell growth rings/layers are produced (Gurney et al., 2005). All shells used in this study showed clear oscillations in OSI ratio, which reflected the seasonal temperature cycle of shallow Patagonian waters. The average temperature inferred from the $\delta^{18}\text{O}$ profile



FIGS. 12–17. Histological sections showing gonadic stages of maturation of *Adelomelon ancilla*. FIGS. 12–14: Females. FIG. 12: Immature gonad with early oogonia (oo); FIG. 13: Developing gonad, with early oocytes (oc) in pre-vitellogenesis process; FIG. 14: Mature gonad, with vitellogenic oocytes. Note the extended cytoplasm with their nucleus (n) and nucleolus (nu); FIGS. 15–17: Males. FIG. 15: Immature gonad; FIG. 16: Developing gonad. Note the spermatogenic tubules (st) developed besides the digestive gland (dg); FIG. 17: Mature gonad. Note the mature sperm into the lumen of spermatogenic tubules and seminal vesicle (sv) evacuating spermatozoa (sp). Scale bars Figs. 12–16 = 50 μ m; Fig. 17 = 100 μ m.



FIGS. 18–23. Size (SL) at which half of the female (FIG. 18) and male (FIG. 19) individuals exhibit matures gonads (SL₅₀). Percentage of gonad maturation vs. shell length in females (FIG. 20) and males (FIG. 21). Relation between shell length (SL) and shell free wet body mass (SFWM) and size at first gonadic maturation of females (FIG. 22) 85 mm (perpendicular bar) and males (FIG. 23) 86 mm (perpendicular bar) of *A. ancilla*.

in *A. ancilla* shells, 10.2°–17.5°C, coincided with historic annual temperature range reported in the zone (9.7°–18.3°C) (Rivas & Beier, 1990). Furthermore, all the shells studied showed two growth marks per year; the first mark was produced in late winter coinciding with the $\delta^{18}\text{O}$ maximum, and the second mark was formed in

late summer in concordance with the $\delta^{18}\text{O}$ minimum. This semiannual pattern of shell marks was also observed in the volutid *Odontocymbiola magellanica* (Gmelin, 1791) (Bigatti et al., 2007), a sympatric species of *A. ancilla* in Golfo Nuevo. However, in other studied volutids, as *Zidona dufresnei* (Donovan, 1823) (Giménez

et al., 2004), *Adelomelon brasiliiana* (Dillwyn, 1817) (Cledón et al., 2005a), as well as in the olivid *Olivancillaria deshayesiana* (Ducros de Saint Germain, 1857) (Arrighetti et al., 2012), all from northern Argentinean coastal waters (temperature ranges from 22°C in late summer to 11°C in late winter), marks are deposited annually. These differences in patterns in shell mark deposition evidenced by Patagonian and species from northern waters in Argentina, might be related to inhabiting different biogeography regions (Argentinean and Magallanic provinces) (Bigatti et al., 2007).

Age, Growth, Production, Mortality and Reproductive Maturity

According to isotopic data and the internal growth marks observed in *A. ancilla* we determined that this species fits to a Gompertz growth model, reaching up to 18 years at 150 mm SL in the Golfo Nuevo region. The long lifespan of *A. ancilla* is comparable to other large gastropods, in particular other Argentine volutid species, for example, 20 years in a 198 mm SL *O. magellanica* individual (Bigatti et al., 2007), 17 years in a 198 mm SL *Z. dufresnei* individual (Giménez et al., 2004), 20 years in a 170 mm SL *A. brasiliiana* individual (Cledón et al., 2005a) and 29 years in a 380 mm SL *A. beckii* (Broderip, 1836) individual (Arrighetti et al., 2011).

The Gompertz growth function proposed for *A. ancilla* also fits to *Z. dufresnei* and *O. magellanica*, whereas for *A. beckii* and *A. brasiliiana* the function that best fitted the size-age data pair was the Von Bertalanffy and the Logistic, respectively (Giménez et al., 2004; Cledón et al., 2005a; Bigatti et al., 2007; Arrighetti et al., 2011). The differences between these functions could be inherent to the particular growth pattern and shell morphology that each species exhibits (Cledón et al., 2005a; Arrighetti et al., 2011), and could reflecting different growing patterns. The species that grow and mature faster could be more suitable to commercial exploitation. Moreover, the overall growth performance of *A. ancilla* ($P = 5.84$) (Fig. 5) was minor than the values referring to other volutids and large gastropods species that are fishery resources in temperate regions along the world as shown in the auximetric grid (Fig. 10) (De Jesús-Navarrete, 2001; Giménez et al., 2004; Cledón et al., 2005a; Bigatti et al., 2007; Arrighetti et al., 2011). Individual somatic production (weight increment per year) of *A. ancilla*

was very low (maximum of 7.4 g SFWM at 120 SL) compared with the other studied volutids (Giménez et al., 2004; Cledón et al., 2005a; Bigatti et al., 2007; Arrighetti et al., 2011). This fact agrees, and it could be associated with the low (40%) foot output (% of foot weight consumable from total SFWM) recorded by Bigatti & Ciocco (2008). Moreover, the relationship between shell weight and SFWM in *A. ancilla* was higher (the shell is thick and heavy) than in the remaining volutids (shells are thinner and slight) (Zabala, 2013). The size-frequency distribution reported here is based on data collected from SCUBA diving by us (SZ and GB). Smaller individuals were not register due to the cryptic condition of juveniles, which live buried in the soft substratum and feed on carrion (Zabala, 2013). The cryptic condition of juveniles could protect them for predators; however, predation was not reported in *A. ancilla* for juveniles nor egg capsules, as it was in other volutids from northern Patagonia (Bigatti et al., 2007; Roche et al., 2013). The lack of juveniles in the sampling is clearly indicated by the size-converted catch curve, where individuals smaller than 12 years of age are under-represented. Therefore, estimates of production and productivity based on this distribution would underestimate true population values, but represents the mature population under study.

In respect to the first reproductive maturity size of *A. ancilla* obtained by gonad histological section, males reached maturity at a smaller size (beginning at 68 mm, with $SL_{50} = 73.5$ mm) than females (beginning at 93 mm, with $SL_{50} = 93.5$ mm). The early male maturation also occurs in other volutids, such as *Z. dufresnei*, *O. magellanica* and *A. brasiliiana* (Giménez & Penchaszadeh, 2003; Cledón et al., 2005b; Bigatti et al., 2008). This difference in size at maturation might indicate that females need more time to be capable of reproduction (Cledón et al., 2005b). Other gastropods also show differences between sexes, for example, *Buccinum cyaneum* Bruguière, 1792 (Miloslavich & Dufresne, 1994) and in some buccinids from Japanese waters, in which males mature at a smaller SL than the females, indicating a more gradual process of maturation (Ilano et al., 2003) associated with a greater reproductive investment in females (Averbuj et al., 2010). Furthermore, the early maturation in males would allow matings between mature females, providing reproductive success of the population. In this sense, if the species is exploited females sizes captured must be bigger than the

SL₅₀, permitting some reproductive seasons that add individuals to the population.

The age at which 50% of *A. ancilla* individuals present gonadic maturation corresponded to 7 and 9 years, for males and females respectively. The late gonadic maturation in comparison with other caenogastropods from other latitudes (Carrick, 1980; Peralta, 2012) occurs also in other volutids and at similar sizes (e.g., *O. magellanica*: 90 mm SL at 8 years and 80 mm SL at 7 years; *Z. dufresnei*: 157 SL and 150 SL at 8 to 9 years both, for females and males respectively). The gonadic maturation of South Atlantic volutids seems to be rather late compared with other large gastropods from northern regions (temperate and tropical species), such as *Gazameda gunni* (Reeve, 1849) (2.5–3 years; Carrick, 1980), but similar to others like *Buccinum undatum* Linnaeus, 1758 (6 years; Gendron, 1992), *Strombus costatus* Gmelin, 1791, and *S. gigas* Linnaeus, 1758 (5 and 7 years, respectively; Wefer & Killingley, 1980).

The relationship between SFWM and SL is not a useful parameter of maturity in *A. ancilla*. In females, the rate change in the increased weight (break point) with respect to shell length was recorded at 84 mm. This increment in body mass is registered before gonadic maturity (93.5 mm) and could be attributable to an early investment in somatic growth, required for a subsequent reproductive investment (Averbuj et al., 2010) or is related to mass increase due to the development of the reproductive accessory glands (Cledón et al., 2005a). Penis length does not appear to be a good indicator of maturity, despite showing a significant positive correlation with size. The penis structure in *A. ancilla* males developed prior to gonad maturation, because all males over 60 mm in SL had a conspicuous penis.

Ecological Importance and Fisheries Suggestions

Small-scale fisheries, and particularly benthic invertebrate fisheries, have played an important role in the development of new fishery management principles and tools (Leiva & Castilla, 2002). Large marine gastropod landings of 1,000 tons/year were recorded in Argentina (Ministerio de Agricultura, Ganadería y Pesca, 2012). Nevertheless, there are few studies that detail the exploitation practices and fishery management. For some marine gastropods (e.g., *Concholepas concholepas* (Bruguère, 1789) in Chile and the abalone *Haliotis* spp. in

Baja California; Leiva & Castilla, 2002), due to their high economic value and excessive capture, the populations have shown serious problems of overexploitation (Tegner, 1989; Castilla, 1996; Ponce-Díaz et al., 1998; Hobday et al., 2001). Recently, Torroglosa & Giménez (2010) stated that *Z. dufresnei* presented a smaller size at reproduction maturation (131 mm and 128 mm for females and males respectively) on the same gastropod population ten years after exploitation. These authors mention that the decrease in the size at first maturity might be a response to resource depletion, caused by overfishing of the slow growing gastropods that occurs in the fishing zone, north of where our study was performed.

The ecological significance of *A. ancilla* in Patagonian shallow waters community is defined by its trophic position among the top benthic predators in the food web it inhabits (Zabala et al., 2013). Studies of *A. ancilla* populations showed extremely low densities at different sites, 0.007 and 0.037 ind/m² in the studied area and 0.007 ind/m² in offshore southern Atlantic Ocean (Zabala, 2013). All features of the population dynamics of *A. ancilla*, such as its longevity, low somatic production, low density, high trophic position and late reproductive maturity including direct development with few embryos inside the egg capsules (Zabala, 2013), place this species as a not recommendable resource for commercial exploitation in Golfo Nuevo populations. Nevertheless, *Adelomelon ancilla* is usually confounded with the sympatric *Odontocymbiola magellanica* due to their external shell similarity, as occurs in the Chilean marine gastropod artisanal fisheries (Osorio, 2002), and could be fished as by catch or target species in open ocean waters if commercial volutid fisheries proliferate. For this reason, and due to *A. ancilla* presents lower populations than *O. magellanica* in shallow waters of Golfo Nuevo (Zabala, 2013), we do not recommend its exploitation in the studied zone. However, in case that this species is exploited commercially in other places, we suggest a minimum size of 100 mm SL for both sexes. Thus, it would ensure that females (LT₅₀: 93.5 mm) can reproduce at least two seasons before harvest ensuring the reproductive success of the species.

Our results should be considered when developing an appropriate management of the resource, in concordance with the reproductive (Penchaszadeh et al., 2009) and ecological parameters (Zabala et al., 2013) of this vulner-

able species. The growth model proposed here should also be useful to integrate management and conservation policies (Leiva & Castilla, 2002).

In conclusion, the volutid *Adelomelon ancilla* appears to be more fragile and susceptible to overexploitation than other studied volutids. For this reason, conservation management policies should be defined carefully.

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MOLECULAR PHYLOGENY AND BIOGEOGRAPHY OF A HIGH MOUNTAIN BIVALVE FAUNA: THE SPHAERIIDAE OF THE TIBETAN PLATEAU

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ABSTRACT

Sphaeriids represent a highly diverse family of small bivalves inhabiting freshwater habitats worldwide. They are recognized for their high dispersal capacity as well as for a high capability to adapt to such extreme environments as high mountain regions over 4,000 m above sea level (a.s.l.). The Tibetan Plateau, located in central Asia, is such a high elevation area. Here, we present the first broad spatial sampling of sphaeriids from the Tibetan Plateau, which allowed us to describe the Recent fauna as well as their biogeographical background. Our study revealed six sphaeriid species inhabiting various habitats on the Tibetan Plateau: *Pisidium nitidum*, *P. stewarti*, *P. subtruncatum*, *P. zugmayeri*, *Pisidium* sp. I and *Musculium kashmirensis*. Four species are newly recorded for this region. Based on phylogenetic analyses using molecular information from three different genes (COI, 16S and 28S) we conclude that the extant diversity of Tibetan Plateau sphaeriids might have been caused by multiple colonization events. Furthermore, the zoogeographic affinities of Tibetan Plateau sphaeriids are mostly related to the Palearctic. Similar patterns have been observed in previous studies of other freshwater molluscan taxa in the area.

Key words: *Pisidium*, *Musculium*, Himalayas, dispersal, molecular phylogeny, colonization.

INTRODUCTION

The family Sphaeriidae comprises small to minute freshwater bivalves with shell lengths ranging from 2.5 to 25 mm (Korniushin, 2006). They are the second most diverse family of freshwater bivalves after the Unionidae, with an estimated number of 227 species (Graf, 2013) in five genera: *Eupera* Bourguignat, 1854; *Byssanodonta* d'Orbigny, 1846; *Sphaerium* Scopoli, 1777; *Musculium* Link, 1807; and *Pisidium* C. Pfeiffer, 1821 (Dreher Mansur & Meier-Brook, 2000; Korniushin & Glaubrecht, 2002). However, phylogenetic relationships among and within the sphaeriid taxa are still largely unresolved (Lee & Ó Foighil, 2003; Korniushin & Glaubrecht, 2006, and references therein; Schultheiss et al., 2008), which results in conflicting classifications especially on the generic level (Graf et al., 2013).

Sphaeriids are currently distributed worldwide (except for Antarctica; Bogan, 2008) and are known for their high capability to adapt to diverse habitats and extreme environments, such as high mountain regions > 4,000 m above sea

level (a.s.l.) (Preston, 1909; Prashad, 1933; Bössneck, 2012). Furthermore, sphaeriid species may have a large distribution range (i.e., transcontinental distribution), but can also be restricted to small ranges (Korniushin, 2006).

These characteristics as well as their high passive dispersal capacity (Kappes & Haase, 2012) make the Sphaeriidae suitable model organisms for phylogeographic studies, particularly in species-poor high-elevation regions such as the Tibetan Plateau.

The Tibetan Plateau of central Asia is the largest and highest plateau on earth (mean elevation of ~5,000 m a.s.l.; Royden et al., 2008) and surrounded by high mountain ranges: Qilian and Altun Mountains in the north, Himalaya and Karakorum Mountains in the south and west, and Longmen Mountains in the east (Chang et al., 1989). Approximately 1,600 freshwater and saline lakes greater than 1 km² are scattered throughout the plateau (Zheng, 1997). These lakes are typically oligotrophic (Xing et al., 2009), and many of them are part of endorheic drainage basins (Revenga et al., 1998). Besides the lakes, numerous smaller lentic water bodies,

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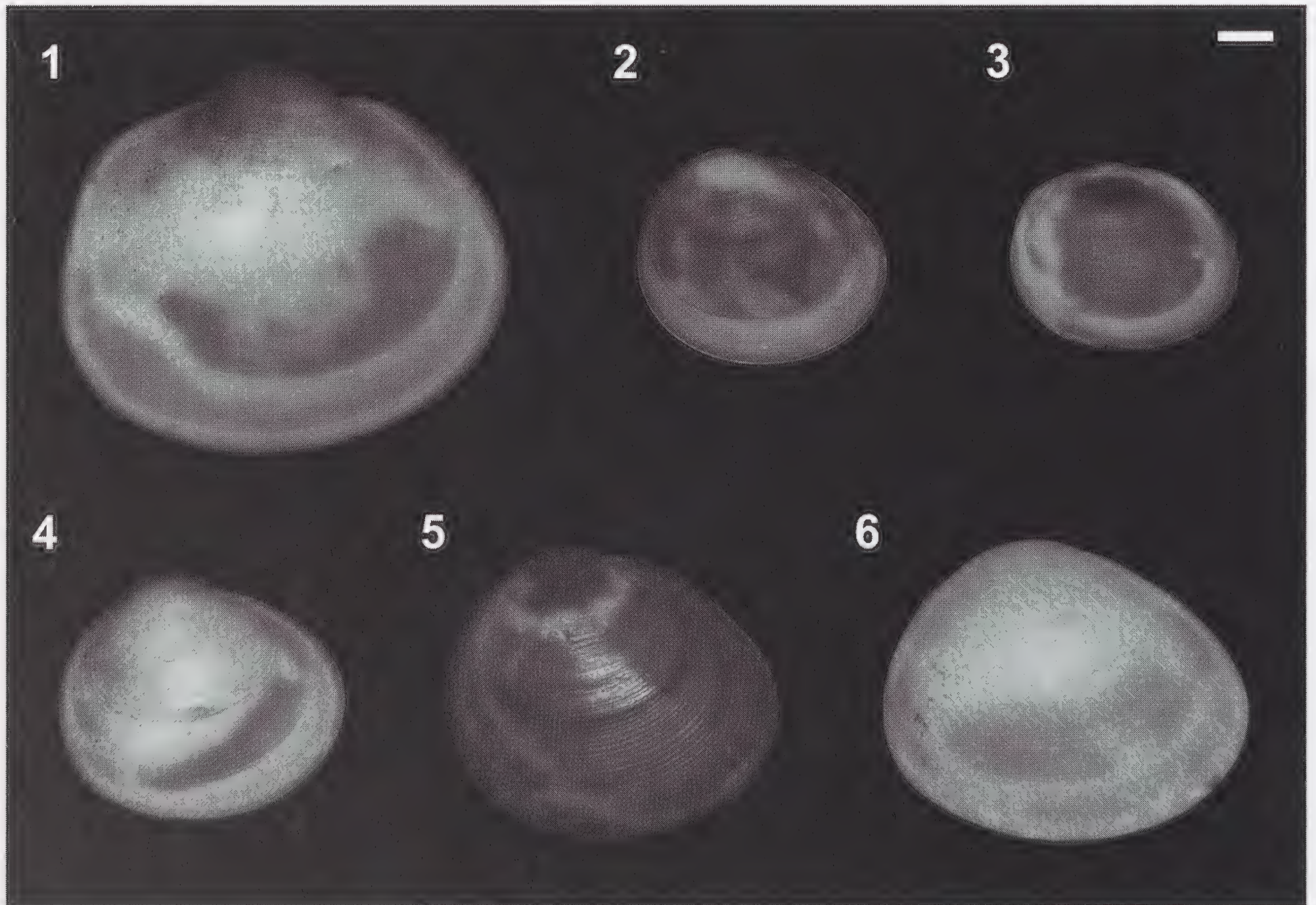
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such as ponds and wetlands, are present (An et al., 2009). The plateau with its large glaciers ("third pole"; Xu et al., 2009) is of great importance for regional freshwater systems, because several major rivers of South and East Asia (such as the Yangtze, Mekong, Brahmaputra, and Indus) have their headwaters in this area.

The faunal components of the Tibetan Plateau are mostly thought to be of Palearctic and Oriental origin (Yang et al., 2009), and the freshwater mollusk fauna seems to be mainly characterized by Palearctic species (Oheimb et al., 2011, 2013). Thereby, the Himalayan mountain range forms the southern border to the Oriental region (Cox, 2001; Morrone, 2002) and is assumed to be a major dispersal barrier for freshwater taxa (Bănărescu, 1992; Abell et al., 2008; Oheimb et al., 2013). In addition, extant patterns of diversity and distribution of species throughout the Tibetan Plateau are mainly influenced by extreme geological changes during the plateau's uplift, as well as climate fluctuations especially during the Pleistocene (e.g., Last Glacial Maximum, ca. 20,000 years ago;

Yang et al., 2009). Although the Tibetan Plateau was probably not completely covered by an ice sheet throughout the Pleistocene (Zheng & Rutter, 1998; Shi, 2002), the unstable climatic conditions resulted in massive environmental changes over time, such as lake-level fluctuations (Lehmkuhl & Haselein, 2000; Dietze et al., 2010). These changing environments might have triggered diversification processes in Tibetan Plateau freshwater organisms, though the regional freshwater fauna and particularly the mollusk fauna remains species-poor and scarcely studied. A total of three gastropod genera (*Radix* Montfort, 1810; *Gyraulus* Charpentier, 1837; *Valvata* O. F. Müller, 1773) and two genera of bivalves (*Musculium*, Fig. 1; *Pisidium*, Figs. 2–6) were reported for the plateau (Prashad, 1933; Mischke et al., 2010; Bössneck, 2012; Oheimb et al., 2011).

Two recent studies focused on the biogeographical history of pulmonate freshwater gastropods (the lymnaeid genus *Radix* and the planorbid genus *Gyraulus*) on the Tibetan Plateau (Oheimb et al., 2011, 2013).



FIGS. 1–6. Sphaeriid species sampled across the Tibetan Plateau. FIG. 1: *M. kashmirensis* (I), location CN19; FIG. 2: *P. stewarti* (VI), CN07; FIG. 3: *Pisidium* sp. I (II), CN07; FIG. 4: *P. nitidum* (III), CN21; FIG. 5: *P. subtruncatum* (IV), CN14; FIG. 6: *P. zugmayeri* (V), CN22. Roman numerals refer to the respective phylogenetic lineages, see Fig. 12. Scale bar = 0.5 mm.

Although sphaeriid clams often occur in sympatry with *Radix* spp. and *Gyraulus* spp., major biological and ecological differences exist, potentially affecting phylogeographical patterns. First, the clams are soft-substratum-dwelling filter-feeders with a potential to influence ecosystem processes in freshwater systems (Vaughn & Hakenkamp, 2001; Dillon, 2004). Secondly, sphaeriid species show the reproductive strategy of euviviparity, that is, eggs, embryos, and juveniles are bred in specialized structures of the parental gills (Korniushin & Glaubrecht, 2006), providing a comparatively stable environment for the offspring. Thirdly, the (passive) dispersal capacity of sphaeriids is presumed to be very high (Mackie, 1979; Schultheiss et al., 2008), thus playing a major role in colonizing new habitats (Zelaya & Marinone, 2012). In fact, there are several mechanisms enabling these small bivalves to disperse between waterbodies in close proximity, as well as over long distances (Kappes & Haase, 2012). Vector-mediated passive dispersal seems to be of particular

importance. For example, clams were found being attached to the limbs of freshwater insects and amphibians (aquatic vectors; Rees, 1965; Bilton, 2001) and to the feathers and legs of different waterbirds, as well as to the extremities of flying insects (aerial vectors; Wesselingh et al., 1999; Figuerola & Green, 2002; Zelaya & Marinone, 2012). Furthermore, Mackie (1979) reported that juvenile sphaeriids are able to survive the gut passage in ducks.

Considering these particular characteristics of Sphaeriidae (in contrast to other Tibetan Plateau freshwater mollusks) as well as the special environmental conditions of the high-elevation Tibetan Plateau, this study has two major aims:

- (I) Assessment of the Tibetan Plateau Sphaeriidae fauna (including data about diversity and ecology) based on extensive sampling.
- (II) Reconstruction of the biogeographical background of the Sphaeriidae species inhabiting the plateau (e.g., number of colonization events, zoogeographic affinities) based on the phylogenetic information inferred from three different DNA markers.

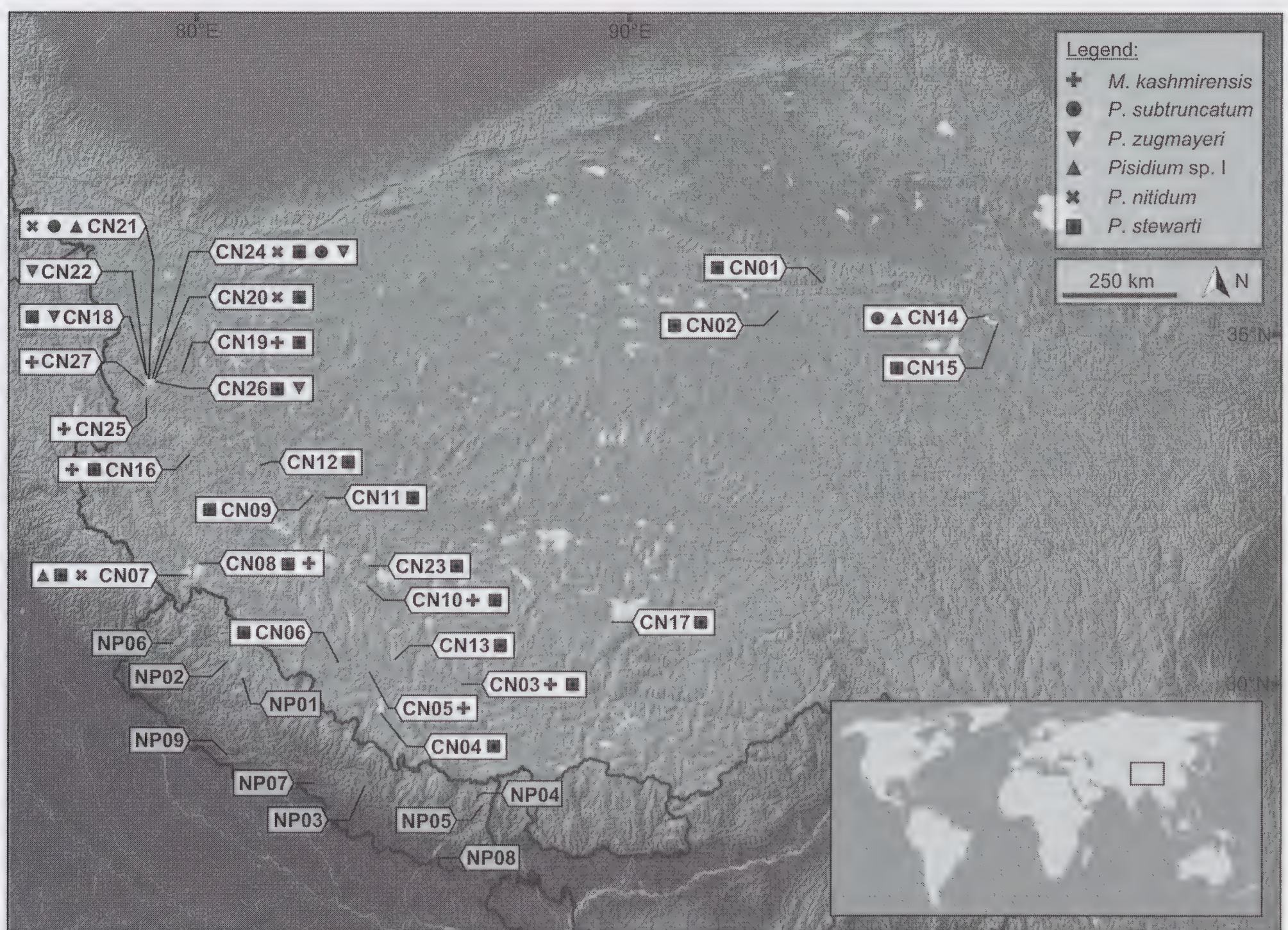


FIG. 7. Sampling sites of Sphaeriidae across the Tibetan Plateau and Nepal. Locality information is given in the Supplementary material (Appendix 1).

MATERIAL AND METHODS

Sampling

During field work conducted between 2009 and 2012, specimens of the family Sphaeriidae were found at 27 locations throughout the Tibetan Plateau (Fig. 7). Specimens were collected by sieving bottom substrate of several types of freshwater habitats, mostly lakes, rivers, ponds, and wetlands (Figs. 8–11) and preserved in 80% ethanol. For comparison, additional sphaeriid specimens were included from 39 localities in parts of Asia (especially locations in the south of the Himalayas, six species from Nepal), Europe and Africa (Appendix 1).

Species determination was performed based on shell morphology (soft tissue removed by boiling in 3% sodium hydroxide solution) and soft body anatomy. Vouchers (DNA, shells) are stored at the Systematics and Biodiversity collection of the University of Giessen (UGSB;

Appendix 1). For morphologically determined sphaeriids from some Tibetan Plateau locations, no molecular data could be obtained (Appendix 1).

Given the conflicting taxonomic concepts among Sphaeriidae (Graf, 2013), we here use the traditional nomenclature on species level (according to Korniuschin & Glaubrecht, 2002), while classification of the clades inferred is based on the phylogenetic analyses of Lee & Ó Foighil (2003). Acknowledging that the purpose of this paper is not primarily taxonomic, future studies based on more comprehensive global datasets should address the internal classification of Sphaeriidae.

DNA Isolation, PCR, and Sequencing

This study includes new genetic data for a total of 101 specimens, including four specimens of *Eupera* spp. as outgroup (Appendix 1). Genomic DNA was extracted from individual



FIGS. 8–11. Representatives of the four dominant habitat types inhabited by sphaeriid species on the Tibetan Plateau. FIG. 8: Wetland (location CN05); FIG. 9: Lake (CN21); FIG. 10: River (CN26); FIG. 11: Pond (CN01).

clams using the CTAB protocol described by Wilke et al. (2006). For phylogenetic analyses, we obtained sequences from fragments of three genes: (1) mitochondrial cytochrome oxidase *c* subunit I (COI), (2) mitochondrial large ribosomal subunit (mtLSU rRNA or 16S) and (3) nuclear large ribosomal subunit (nLSU rRNA or 28S). Standard and modified primers were used for the amplification of COI: LCO1490 (Folmer et al., 1994) or a modified version (lacking 5 bases from the 5' end) and a modified version of HCO2198 (Folmer et al., 1994; minus the first 6 bases at the 5' end). The latter has already been used before especially for freshwater bivalves (Hoeh et al., 2002). Forward and reverse primers for 16S were taken from Palumbi et al. (1991; 16Sar-L and 16Sbr-H) and for 28S from Park & Ó Foighil (2000; D23F and D6R).

PCR cycling conditions were as follows: an initial denaturation step at 95°C for 60 s, followed by 30–36 amplification cycles (denaturation at 95°C for 30 s, annealing at 52°C for 30 s, and elongation at 72°C for 30 s), depending on the concentration of genomic DNA. The reaction was terminated after a final extension step at 72°C for 180 s. Sequences (forward and reverse) were visualized on an ABI 3730 XL sequencer (Life Technologies) using a Big Dye Terminator Kit (Life Technologies).

In addition, 16S sequences from 49 sphaeriid specimens were obtained from GenBank (Appendix 2).

Sequence Alignment

The alignments of sequences were done individually for all three fragments (68 COI, 146 16S, and 32 28S). The first base pairs (bp) behind the 3' end of each primer were difficult to read in all fragments. Therefore, we trimmed each sequence, leaving a 599 bp-long COI fragment, a 741–743 bp-long 28S fragment, and a 472–503 bp-long 16S fragment. The COI and 28S sequences could be unambiguously aligned using ClustalW implemented in BioEdit 7.0.8.0 (Hall, 1999). The alignment of 16S sequences was performed following the instructions for structural alignment by Kjer et al. (2009) based on the LSU rRNA structure model of *Mytilus edulis* (Hoffmann et al., 1992). In total, three regions were excluded from the 16S dataset because no reliable alignment could be achieved (positions 66–80, 281–296, and 341–354), leaving a final alignment of 471 bp (the aligned dataset is available from the corresponding author upon request).

Phylogenetic Analyses

The subsequent analyses (except network analyses) were performed using all 119 unique haplotypes of a combined dataset of COI, 16S, and 28S sequences.

The COI, 16S, and 28S partitions were separately tested for substitution saturation using DAMBE 5.2.73 (Xia et al., 2003; Xia & Lemey, 2009). Under the assumption of a symmetrical as well as an asymmetrical tree, the test showed only little saturation for all three partitions.

Phylogenetic relationships were reconstructed using Bayesian inference implemented in the software package MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001; parameters: nchains = 4, ngen = 8,000,000, samplefreq = 20, temp = 0.1). Prior to the analyses, jModelTest 0.1.1 (Posada, 2008) was used to find the best fit model of sequence evolution for the individual datasets based on the corrected Akaike Information Criterion (AICc). For the COI partition the program selected the HKY+I+G model and for the 28S partition the TIM3+I+G model. Two different models were applied for 16S based on the structural alignment: the 16B model (Schöniger & Haeseler, 1994) for the stem regions and the HKY+G model for the loop regions.

Monitored in Tracer v1.5.0 (Drummond & Rambaut, 2007), the combined set of trees showed both high ESS (effective sample size) values (> 300 for all parameters) and a smooth frequency plot. For the consensus tree the first 25% of the sampled trees (N = 100,000) were discarded as burn-in.

Phylogeographical Analyses

We performed a parsimony network analysis for the most widespread and most abundant species *P. stewarti* using TCS 1.21 (Clement et al., 2000) and a combined dataset of COI and 16S sequences. The resulting dataset contained 14 specimens from nine locations across the Tibetan Plateau.

RESULTS

Diversity, Distribution and Ecology

A total of six sphaeriid species were sampled across the 27 localities on the Tibetan Plateau including five species of *Pisidium* – *P. stewarti* Preston, 1909; *P. subtruncatum* Malm, 1855;

P. zugmayeri Weber, 1910; *P. nitidum* Jenyns, 1832; *Pisidium* sp. I – and one species of *Musculium*, *M. kashmirensis* (Prashad, 1937) (Figs. 1–6). *Pisidium* sp. I represents an undetermined but morphologically distinct species from the clade *Cyclocalyx*. The Tibetan Plateau taxa occurred in different habitats (Table 1). Only specimens of *P. stewarti* and *M. kashmirensis* inhabited all major habitat types: wetlands, lakes, rivers and ponds. Lakes were the most species-rich habitat type (five species). The most frequent species on the Tibetan Plateau was *P. stewarti*, found at 21 out of 27 locations (77.8%). With a frequency of 29.6% (found at eight locations), *M. kashmirensis* was the second most common species. The remaining species were relatively rare: *P. nitidum* and *P. zugmayeri* were each observed at four different locations (14.8%); *P. subtruncatum* and *Pisidium* sp. I were collected at three locations (11.5%). At 12 sampling sites (Table 1) two or more sphaeriid species occurred in sympatry (up to four at the same location). A total of seven different species assemblages were found. The most frequent composition was *M. kashmirensis* together with *P. stewarti* (in 5 out of 12 locations; Fig. 7).

General distribution data of Tibetan Plateau sphaeriids were retrieved from the literature and the present sampling (Table 2). *Pisidium nitidum* and *P. subtruncatum* are both known to have a large distribution range inhabiting several parts of the Holarctic; *P. zugmayeri* is probably endemic to the Tibetan Plateau. The Recent distribution of *P. stewarti* is restricted to areas with cold climates in central Asia and Siberia.

Musculium kashmirensis is distributed in Kashmir, Nepal and on the Tibetan Plateau. Note that another species, *M. goshaitanensis* Nesemann & Sharman, 2005, was described from Punyamata, Kathmandu, Bagmati, Nepal.

Phylogenetic Analyses

The Bayesian analysis revealed a tree with five major clades (Fig. 12), which correspond to the major lineages of Lee & Ó Foighil (2003): *Sphaerium*, *Pisidium* s. str., *Cyclocalyx*, *Odhneripisidium* and *Afropisidium*. The topology is unresolved for basal as well as for few terminal nodes. Four of the major clades are well supported with a Bayesian Posterior Probability (BPP) ≥ 0.95. The genus *Pisidium* appears paraphyletic in the phylogeny as the clade comprising *Pisidium* s. str., including specimens of *P. amnicum* (O. F. Müller, 1774) and *P. dubium* (Say, 1817), forms the sister clade of the *Sphaerium* clade, whereas *Cyclocalyx* is the sister group to the former two clades.

The six sphaeriid species of the Tibetan Plateau do not form a monophyletic group and belong to the clades of *Sphaerium*, *Cyclocalyx* and *Odhneripisidium*. The clades of *Afropisidium* and *Pisidium* s. str. are not present on the Tibetan Plateau. The phylogenetic relationships of the Tibetan Plateau sphaeriids to their respective sister groups are described as following (Fig. 12):

- (I) The Tibetan Plateau *M. kashmirensis* are not monophyletic but form a well-supported but unresolved clade (BPP

TABLE 1. Sphaeriid species found among sampling sites on the Tibetan Plateau. N_S = number of species per habitat type; N_L = number of locations where the respective species was found; F = frequency of species; Habitat Types: wetlands, lakes, rivers, ponds ('others' are described in detail in Appendix 1); CN01–CN27 = location codes (see also Fig. 7), * = sympatry occurring.

Species	Wetlands						Lakes					Rivers					Ponds					others				N _L	F (%)		
	CN03*	CN04	CN05	CN11	CN12	CN18*	CN22	CN07*	CN13	CN10*	CN15	CN17	CN21*	CN02	CN16*	CN19*	CN26*	CN27	CN01	CN06	CN08*	CN09	CN23	CN14*	CN20*			CN24*	CN25
<i>M. kashmirensis</i>	x	x								x					x	x		x			x						x	8	29.6
<i>P. nitidum</i>								x				x													x	x		4	14.8
<i>P. stewarti</i>	x	x		x	x	x		x	x	x	x	x		x	x	x	x		x	x	x	x	x		x	x		21	77.8
<i>P. subtruncatum</i>												x													x		x	3	11.1
<i>P. zugmayeri</i>						x	x										x									x		4	14.8
<i>Pisidium</i> sp. I								x				x													x			3	11.1
N _S	3						5					3					2					6							

TABLE 2. Species of the family Sphaeriidae collected on the Tibetan Plateau with recent distribution and range type (except for *Pisidium* sp. I) based on the literature and own data.

Species	Recent distribution		Range type
<i>M. kashmirensis</i>	(Prashad, 1937)	Tibetan Plateau ¹ , Kashmir ² , Nepal ¹	restricted
<i>P. nitidum</i>	Jenyns, 1832	Holarctic ³	widespread
<i>P. stewarti</i>	Preston, 1909	central Asia, north of the Himalayas ^{4,5,6}	restricted (to cold climates)
<i>P. subtruncatum</i>	Malm, 1855	Holarctic ³	widespread
<i>P. zugmayeri</i>	Weber, 1910	Tibetan Plateau ^{7,8}	endemic

¹this study; ²Prashad, 1937; ³Kuiper et al., 1989; ⁴Preston, 1909; ⁵Bogan, 2011; ⁶Bössneck, unpubl. data; ⁷Prashad, 1933; ⁸Mischke et al., 2010

- 1.00) with *M. kashmirensis* from Nepal, *M. japonicum* (Westerlund, 1883) from Japan and *M. miyadaii* Mori, 1993, from Russia (Kuril Islands). The relationship of this clade to *M. lacustre* (O. F. Müller, 1774) from Germany, Georgia and the U.S.A., is likewise unresolved (BPP 0.96).
- (II) *Pisidium* sp. I (clade *Cyclocalyx*) is not monophyletic but clusters with *P. casertanum* (Poli, 1791) from Germany (BPP 0.68). The sister of this clade is a specimen of *P. atkinsonianum* Theobald, 1876 from Nepal (BPP 0.62). Note also that *P. casertanum* is not monophyletic according to the phylogeny of the present study.
- (III) The Tibetan Plateau specimens of *P. nitidum* (clade *Cyclocalyx*) form no monophyletic group but cluster together with other *P. nitidum* specimens and specimens of three other species (*P. edlaueri* Kuiper, 1960; *P. milium* Held, 1836; and *P. pseudosphaerium* J. Favre, 1927) from Germany, Macedonia and Turkey in an unresolved clade (BPP 0.52).
- (IV) The Tibetan Plateau *P. subtruncatum* (clade *Cyclocalyx*) are monophyletic (BPP 1.00) and sister to other specimens of this species from Georgia, Italy and Macedonia, together forming a well supported clade (BPP 0.99).
- (V) *Pisidium zugmayeri* (clade *Cyclocalyx*) forms a monophyletic group (BPP 1.00) that falls into a strongly supported clade of *P. casertanum* from Ireland, Macedonia and Turkey (BPP 0.99); thereby, the specimen from Ireland is the direct sister to the Tibetan Plateau specimens (BPP 1.00).
- (VI) The specimens of *P. stewarti* represent a distinct monophyletic group (BPP 1.00) within the *Odhneripisidium* clade, being sister to *P. moitessierianum* (Paladilhe, 1866) (BPP 0.71).

The genetic distance between *P. stewarti* and its sister group; however, appears to be high as revealed by long branches from their common ancestor. For *P. stewarti* a haplotype network analysis was conducted (Fig. 13).

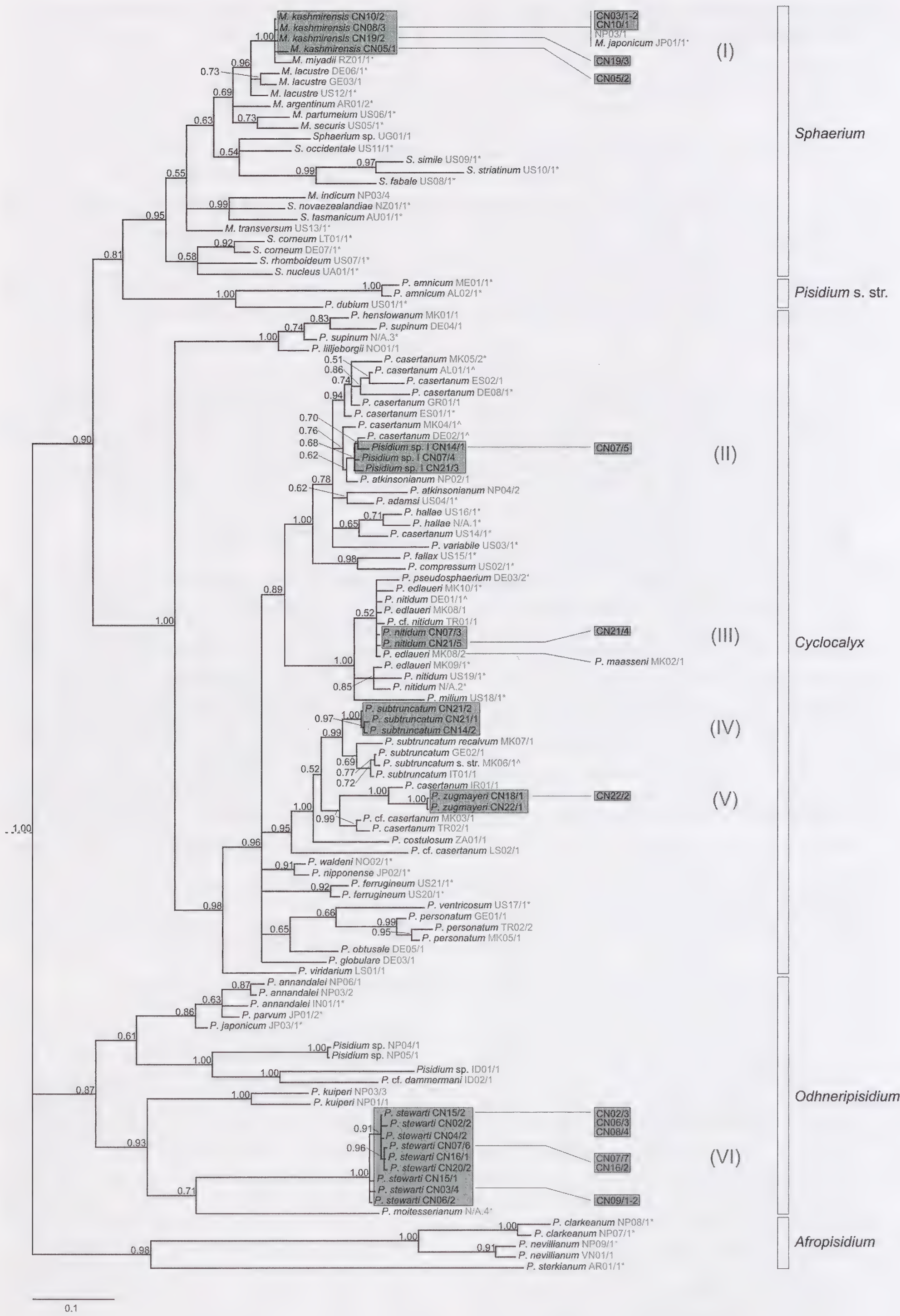
Phylogeographical Analyses

The parsimony network analysis of 14 *P. stewarti* specimens from the Tibetan Plateau revealed nine unique haplotypes for the combined dataset of 16S and COI. These haplotypes fall into two subclades (A: 6 haplotypes, B: 3 haplotypes) separated by eight mutational steps (Fig. 13). Specimens from four geographically very distant locations (max. ca. 1,400 km) share the most frequent haplotype (subclade A in Fig. 13). There is no obvious correlation between genetic distances (mutational steps) and geographical distances on the plateau (map in Fig. 13).

DISCUSSION

Diversity, Distribution and Ecology

Prior to the present study, little was known about sphaeriid distribution in freshwater systems throughout the Tibetan Plateau. Besides the records of *P. zugmayeri* in lakes Bangong Co (western Tibet; Prashad, 1933) and Donggi Cona (eastern Tibet; Mischke et al., 2010), there were only few other records of *P. stewarti* from the plateau (Preston, 1909; Dance, 1967). This study, however, demonstrates that the diversity of the Sphaeriidae from the Tibetan Plateau is significantly higher than previously known, as revealed by the first records of *M. kashmirensis*, *P. subtruncatum*, *P. nitidum* and *Pisidium* sp. I. Additionally, a recent drilling core analysis by Mischke et al. (unpubl. data;



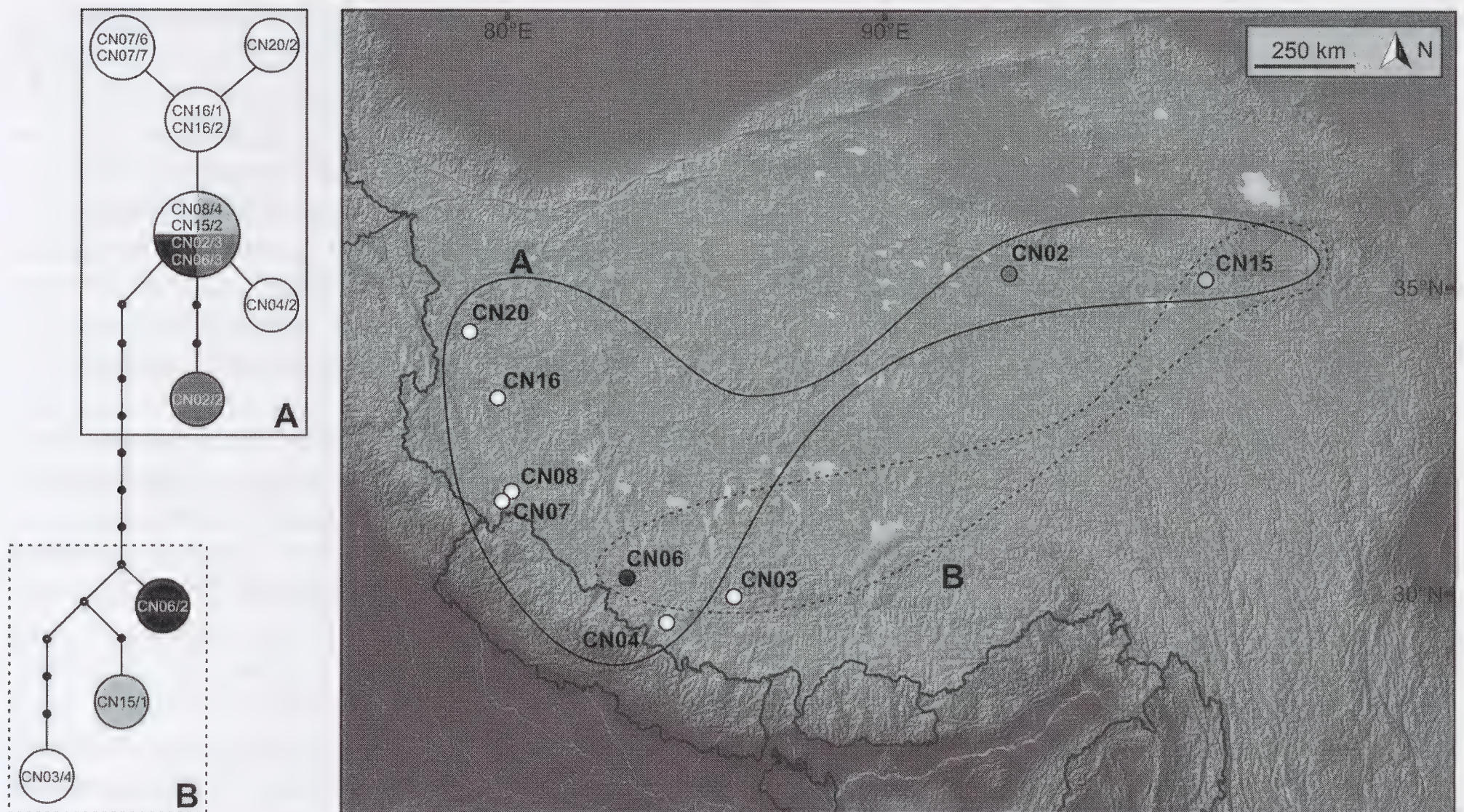


FIG. 13. Statistical parsimony network of *P. stewarti* from the Tibetan Plateau. The geographic distribution of subgroups (A, B) is shown in the map. Locations including more than one haplotype are labeled in different greyscales.

ca. 16,000 years ago; Luanhaizi Lake, Qinghai province, northeastern Tibetan Plateau, China) has revealed shell fragments of *P. obtusale* (Lamarck, 1818). Hence, additional sampling might reveal even more sphaeriid species on the Tibetan Plateau.

Sphaeriids are able to occupy a great variety of habitats, however, the highest species diversity is observed in freshwater lakes, ponds, and small rivers (Korniushin, 2006). Some sphaeriid species show clear preferences to particular habitats (e.g., *P. lilljeborgi* Clessin, 1886: lakes; *P. supinum* Schmidt, 1851: rivers), others are generalists inhabiting a wide range of habitats. Thus, such generalists seem to be less sensitive to changes in environmental conditions

(e.g., oxygen content; Korniushin, 2006). This fact might be of advantage for generalist species occurring in extreme habitats (i.e., high mountain ranges like the Tibetan Plateau) with partial seasonal freezing, increased ultraviolet radiation and low oxygen level. Interestingly, *P. stewarti* and *M. kashmirensis* were present in all major habitat types; the remaining species appear to be more restricted to particular habitats (Table 1). This habitat occupation pattern might be due to an increased specialization of species at the high elevations of the Tibetan Plateau. For instance, *P. nitidum* and *P. subtruncatum* generally inhabit lotic (flowing) as well as lentic (stagnant) waters (Kuiper et al., 1989), but they have been only

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FIG. 12. Bayesian phylogram of Sphaeriidae (comprising the genera *Musculium*, *Sphaerium*, and *Pisidium*) based on COI, mtLSU rRNA (16S), and nLSU rRNS (28S) sequences. Bayesian posterior probabilities (higher than 0.5) are given next to the respective node. Individuals from the Tibetan Plateau are highlighted in gray and consecutively numbered with roman numerals (see also Fig. 1). The scale bar represents substitutions per site according to the applied model of sequence evolution. Major clades are marked with white bars according to the classification of Lee & Ó Foighil (2003). Specimens (sequences) exclusively from GenBank are marked with asterisks (*), such with additional new COI sequence data are marked with circumflex accents (^). Outgroups (*Eupera* spp.), with the specimen codes: MW01/1, NA01/1, and UG01/1–2, are not shown. Specimens sharing a haplotype are provided next to the phylogram except for CN01/1, CN02/1, CN03/3, CN04/1, CN06/1, CN07/1–2, CN08/1–2, CN11/1, CN12/1, CN13/1, CN17/1, CN19/1, and CN20/1, which share the same 16S haplotype with CN15/2 and of which no COI data are available. For detailed specimen information see Appendices 1 and 2.

recorded from lakes on the plateau. However, a sampling artifact cannot be ruled out due to the small sample sizes ($N = \text{max. } 7$) for each habitat type. Nonetheless, the highest species diversity was observed for the habitat type “lakes” (see above), which may be caused by a higher diversity of microhabitats in lakes, or by the larger size and higher stability of lakes compared to ponds and wetlands.

Recent studies on Tibetan Plateau freshwater gastropods have detected three to four distinct genetic lineages for *Radix* (Oheimb et al., 2011), two lineages for *Gyraulus* (Oheimb et al., 2013) and only one lineage for *Valvata* (unpubl. data). Thus, the diversity of Tibetan Plateau sphaeriids seems to be slightly higher. A relatively high species diversity of sphaeriids is also reported for other high mountain ranges such as the Andes in South America, as well as Mt. Kilimanjaro and Mt. Kenya in eastern Africa (Bössneck, 2012). General observations of the freshwater mollusk fauna in high mountainous regions show that the gastropod-dominated fauna at lower elevations is replaced by a sphaeriid-dominated fauna with increasing elevation (Bössneck, 2012). In conclusion, the Sphaeriidae appear to be one of the few freshwater mollusk taxa being well adapted to high mountainous regions.

Biogeography of Tibetan Plateau Sphaeriids

Given that the Tibetan Plateau sphaeriids do not constitute a monophyletic group (Fig. 12), independent colonizations of the plateau have to be assumed. We found at least six independent colonization events; four within the clade *Cyclocalyx*, one for *Odhneripisidium* and one for the clade *Sphaerium*, each representing a single species lineage. Phylogenetic analyses of the Tibetan Plateau freshwater gastropod genera *Radix* and *Gyraulus* revealed at least three and two independent colonization events of the plateau by these taxa, respectively (Oheimb et al., 2011, 2013). Apparently, there are decisive differences between these three mollusk taxa resulting in a potentially higher colonization rate of sphaeriids. One major difference possibly causing the increased colonization rate is the high dispersal capacity in sphaeriids (see Introduction).

In order to infer the biogeographical relationships of the Tibetan Plateau sphaeriid species, knowledge about the zoogeographical affinities of the respective sister groups are useful. Generally, the sister groups are dominated by species from northern Eurasia (Palearctic

region). Based on the general distribution of the respective species (Table 2) and the close relationship to Palearctic taxa, *P. nitidum*, *P. subtruncatum* and *P. zugmayeri* can be considered as Palearctic elements of the Tibetan Plateau fauna. The affinity of *Pisidium* sp. I, however, remains questionable due to the weakly supported relationships to their potential sister taxa, the Palearctic species *P. casertanum* and the species *P. atkinsonianum* (Fig. 12).

In the case of *M. kashmirensis* several specimens from the Tibetan Plateau as well as from Far East Russia and Japan (Palearctic region) cluster with one specimen from the Oriental region of Nepal. In contrast to *Gyraulus* spp. (Oheimb et al., 2013), *M. kashmirensis* may thus have crossed the Himalayan mountain range. However, for *M. kashmirensis* from Nepal we could only generate sequence data from the relatively conservative 16S gene but not from COI. Furthermore, this species has been collected in Nepal only in the highly frequented Kathmandu valley and thus may have been recently introduced there. As the holotype of *M. kashmirensis* was described from Kashmir (Prashad, 1937), a region with both Palearctic and Oriental elements (Das, 1966), the zoogeographical affinity of Tibetan Plateau *M. kashmirensis* remains ambiguous.

The sister species of *P. stewarti* is *P. moitessierianum* in our phylogeny, however, the relationship is characterized by relatively long branches. Although locality information for the respective *P. moitessierianum* specimen was not provided with the original GenBank data, the distribution of this species is known to be restricted to Eurasia (Zettler & Kuiper, 2002), and it thus most likely has a Palearctic origin.

Additionally, Pleistocene fossils of *P. stewarti* described from several parts in Europe (Briggs & Gilbertson, 1980; Ložek, 2001) also suggest Palearctic affinities for this species.

In contrast to other Tibetan Plateau sphaeriids (i.e., *P. subtruncatum* and *P. nitidum* with a Holarctic distribution), *P. stewarti* often occurs on high elevations in central Asia, with a remarkably wide range across the plateau. Therefore, this species was used for a more detailed haplotype network analysis (Fig. 13). The results of this analysis suggest high dispersal rates throughout the Tibetan Plateau. This is illustrated by specimens from geographically very distant locations (i.e., ca. 1,400 km) which share the same haplotype. In contrast, the maximum geographical distance

between locations where identical haplotypes have been found is approx. 450 km and 300 km for *Gyraulus* and *Radix*, respectively (Oheimb et al., 2011, 2013). This observation highlights the high efficiency of sphaeriid dispersal. Nevertheless, the Himalayan mountain range also represents a crucial biogeographical barrier for sphaeriids, as for other Tibetan Plateau freshwater mollusks. This might be exemplified by Palearctic affinities of most of the Tibetan Plateau sphaeriids. Furthermore, this fauna is conspicuously different from the adjacent Oriental Sphaeriidae fauna from Nepal comprising ten different and only one shared species (*M. kashmirensis*) (see also Korniuschin & Bössneck, 2003; Nesemann & Sharma, 2005).

CONCLUSION

The present study demonstrates that the Tibetan Plateau Sphaeriidae fauna is characterized by a previously unknown high species diversity, which is dominated by Palearctic elements. A total of six different species have been recorded, four of them for the first time on the Tibetan Plateau. Furthermore, our study supports the hypothesis that the Himalayan mountain range represents a crucial biogeographical barrier for freshwater mollusks.

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APPENDIX 1. List of studied specimens including specimen code (^ = specimens with 16S from GenBank and COI sequences produced by ourselves), taxon, locality information, voucher details (materials are stored at the Systematics and Biodiversity Collection of the University of Giessen – UGSB), and GenBank accession numbers.

Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen-Bank acc.#	16S Gen-Bank acc.#	28S Gen-Bank acc.#
AL01/1^	<i>Pisidium casertanum</i>	Albania, Qark Korça	40.89623°	20.71040°	UGSB 10544	KF483356	EU559094	
CN01/1	<i>Pisidium stewarti</i>	China, Tibet, pond south of Golmud	35.88329°	94.57995°	UGSB 8391		KF483234	
CN02/1	<i>Pisidium stewarti</i>	China, Tibet, small river south of Golmud	35.43374°	93.60115°	UGSB 8392		KF483235	
CN02/2	<i>Pisidium stewarti</i>	China, Tibet, small river south of Golmud	35.43374°	93.60115°	UGSB 9988	KF483357	KF483236	
CN02/3	<i>Pisidium stewarti</i>	China, Tibet, small river south of Golmud	35.43374°	93.60115°	UGSB 9990	KF483358	KF483237	
CN03/1	<i>Musculium kashmirensis</i>	China, Tibet, wetlands west of Ngamring Co	29.34203°	87.16489°	UGSB 8394		KF483238	
CN03/2	<i>Musculium kashmirensis</i>	China, Tibet, wetlands west of Ngamring Co	29.34203°	87.16489°	UGSB 8395	KF483359	KF483239	
CN03/3	<i>Pisidium stewarti</i>	China, Tibet, wetlands west of Ngamring Co	29.34203°	87.16489°	UGSB 8396		KF483240	
CN03/4	<i>Pisidium stewarti</i>	China, Tibet, wetlands west of Ngamring Co	29.34203°	87.16489°	UGSB 9992	KF483360	KF483241	
CN04/1	<i>Pisidium stewarti</i>	China, Tibet, wetlands near Paiku Co	28.77180°	85.56133°	UGSB 8398		KF483242	
CN04/2	<i>Pisidium stewarti</i>	China, Tibet, wetlands near Paiku Co	28.77180°	85.56133°	UGSB 9995	KF483361	KF483243	
CN05/1	<i>Musculium kashmirensis</i>	China, Tibet, wetlands west of Saga	29.40823°	85.24297°	UGSB 8400		KF483245	

(continues)

(continued)

Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen- Bank acc.#	16S Gen- Bank acc.#	28S Gen- Bank acc.#
CN05/2	<i>Musculium kashmirensis</i>	China, Tibet, wetlands west of Saga	29.40823°	85.24297°	UGSB 8401		KF483246	
CN06/1	<i>Pisidium stewarti</i>	China, Tibet, pond near Lazangxiang	29.50262°	84.57673°	UGSB 8403		KF483244	
CN06/2	<i>Pisidium stewarti</i>	China, Tibet, pond near Lazangxiang	29.50262°	84.57673°	UGSB 9997	KF483362	KF483247	
CN06/3	<i>Pisidium stewarti</i>	China, Tibet, pond near Lazangxiang	29.50262°	84.57673°	UGSB 9998	KF483363	KF483248	
CN07/1	<i>Pisidium stewarti</i>	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 8404		KF483249	
CN07/2	<i>Pisidium stewarti</i>	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 8405		KF483250	
CN07/3	<i>Pisidium nitidum</i>	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 8406	KF483364	KF483251	
CN07/4	<i>Pisidium</i> sp. I	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 8407	KF483365	KF483252	KF483324
CN07/5	<i>Pisidium</i> sp. I	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 8408		KF483253	
CN07/6	<i>Pisidium stewarti</i>	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 10001	KF483366	KF483254	
CN07/7	<i>Pisidium stewarti</i>	China, Tibet, Lake Manasarovar	30.60952°	81.36448°	UGSB 10002	KF483367	KF483255	
CN08/1	<i>Pisidium stewarti</i>	China, Tibet, spring complex north of Lake Manasarovar	30.81483°	81.56236°	UGSB 8409		KF483256	
CN08/2	<i>Pisidium stewarti</i>	China, Tibet, spring complex north of Lake Manasarovar	30.81483°	81.56236°	UGSB 8410		KF483257	

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Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen-Bank acc.#	16S Gen-Bank acc.#	28S Gen-Bank acc.#
CN08/3	<i>Musculium kashmirensis</i>	China, Tibet, spring complex north of Lake Manasarovar	30.81483°	81.56236°	UGSB 8412	KF4833368	KF483258	
CN08/4	<i>Pisidium stewarti</i>	China, Tibet, spring complex north of Lake Manasarovar	30.81483°	81.56236°	UGSB 10003	KF4833369	KF483259	
CN09/1	<i>Pisidium stewarti</i>	China, Tibet, pond near Cam Co	32.08300°	83.66180°	UGSB 8376		KF483260	
CN09/2	<i>Pisidium stewarti</i>	China, Tibet, pond near Cam Co	32.08300°	83.66180°	UGSB 8377		KF483261	
CN10/1	<i>Musculium kashmirensis</i>	China, Tibet, Garing Co	30.76610°	85.01230°	UGSB 8378		KF483262	
CN10/2	<i>Musculium kashmirensis</i>	China, Tibet, Garing Co	30.76610°	85.01230°	UGSB 8379	KF4833370	KF483263	KF483325
CN10/3	<i>Pisidium stewarti</i>	China, Tibet, Garing Co	30.76610°	85.01230°	UGSB 12570		no sequence information	
CN11/1	<i>Pisidium stewarti</i>	China, Tibet, wetlands south of Gerze	32.06443°	83.90559°	UGSB 8383		KF483264	
CN12/1	<i>Pisidium stewarti</i>	China, Tibet, wetlands west of Cakaxiang	32.44139°	82.43805°	UGSB 8384		KF483265	
CN13/1	<i>Pisidium stewarti</i>	China, Tibet, Dajiamang Co	29.63267°	85.74019°	UGSB 8388		KF483266	
CN14/1	<i>Pisidium</i> sp. I	China, Qinghai, artificial dam region near Donggi Cona	35.35419°	98.34894°	UGSB 9087	KF4833371	KF483269	KF483326
CN14/2	<i>Pisidium subtruncatum</i>	China, Qinghai, artificial dam region near Donggi Cona	35.35419°	98.34894°	UGSB 9088	KF4833372	KF483270	KF483327
CN15/1	<i>Pisidium stewarti</i>	China, Qinghai, Donggi Cona	35.28861°	98.70116°	UGSB 9089	KF4833373	KF483267	KF483328
CN15/2	<i>Pisidium stewarti</i>	China, Qinghai, Donggi Cona	35.28861°	98.70116°	UGSB 9090	KF4833374	KF483268	KF483329
CN16/1	<i>Pisidium stewarti</i>	China, Tibet, river west of Ge'gyai	32.45972°	80.89935°	UGSB 10006	KF4833375	KF483271	
CN16/2	<i>Pisidium stewarti</i>	China, Tibet, river west of Ge'gyai	32.45972°	80.89935°	UGSB 10007	KF4833376	KF483272	

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Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen- Bank acc.#	16S Gen- Bank acc.#	28S Gen- Bank acc.#
CN16/3	<i>Musculium kashmirensis</i>	China, Tibet, river west of Ge'gyai	32.45972°	80.89935°	UGSB 12571	no sequence information		
CN17/1	<i>Pisidium stewarti</i>	China, Tibet, river near Nam Co	30.47651°	90.20139°	UGSB 10987	KF483273	KF483355	
CN18/1	<i>Pisidium zugmayeri</i>	China, Tibet, wetlands north of Bangong Co	33.62848°	79.76558°	UGSB 10989	KF483377	KF483275	
CN18/2	<i>Pisidium stewarti</i>	China, Tibet, wetlands north of Bangong Co	33.62848°	79.76558°	UGSB 10461	no sequence information		
CN19/1	<i>Pisidium stewarti</i>	China, Tibet, inflow Bangong Co	33.66933°	80.46586°	UGSB 10990	KF483274	KF483352	
CN19/2	<i>Musculium kashmirensis</i>	China, Tibet, inflow Bangong Co	33.66933°	80.46586°	UGSB 11001	KF483378	KF483276	KF483351
CN19/3	<i>Musculium kashmirensis</i>	China, Tibet, inflow Bangong Co	33.66933°	80.46586°	UGSB 11002	KF483277		
CN20/1	<i>Pisidium stewarti</i>	China, Tibet, dam region near Bangong Co	33.56812°	79.94763°	UGSB 10991	KF483278	KF483353	
CN20/2	<i>Pisidium stewarti</i>	China, Tibet, dam region near Bangong Co	33.56812°	79.94763°	UGSB 10992	KF483379	KF483279	
CN20/3	<i>Pisidium nitidum</i>	China, Tibet, dam region near Bangong Co	33.56812°	79.94763°	UGSB 10396	no sequence information		
CN21/1	<i>Pisidium subtruncatum</i>	China, Tibet, Bangong Co	33.57666°	79.86945°	UGSB 10993	KF483381	KF483281	KF483331
CN21/2	<i>Pisidium subtruncatum</i>	China, Tibet, Bangong Co	33.57666°	79.86945°	UGSB 10994	KF483380	KF483280	KF483332
CN21/3	<i>Pisidium</i> sp. I	China, Tibet, Bangong Co	33.57666°	79.86945°	UGSB 10995		KF483282	
CN21/4	<i>Pisidium nitidum</i>	China, Tibet, Bangong Co	33.57666°	79.86945°	UGSB 10999		KF483285	KF483354
CN21/5	<i>Pisidium nitidum</i>	China, Tibet, Bangong Co	33.57666°	79.86945°	UGSB 11000	KF483384	KF483286	
CN22/1	<i>Pisidium zugmayeri</i>	China, Tibet, wetlands north of Bangong Co	33.62711°	79.77509°	UGSB 10997	KF483382	KF483283	KF483333

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Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen- Bank acc.#	16S Gen- Bank acc.#	28S Gen- Bank acc.#
CN22/2	<i>Pisidium zugmayeri</i>	China, Tibet, wetlands north of Bangong Co	33.62711°	79.77509°	UGSB 10998	KF483383	KF483284	
CN23	<i>Pisidium stewarti</i>	China, Tibet, ponds north of Coqen	31.07629°	84.99603°	UGSB 10230			
CN24/1	<i>Pisidium stewarti</i>	China, Tibet, estuary eastern inflow Bangong Co	33.55763°	79.93183°	UGSB 12596			
CN24/2	<i>Pisidium nitidum</i>	China, Tibet, estuary eastern inflow Bangong Co	33.55763°	79.93183°	UGSB 12597			
CN24/3	<i>Pisidium subtruncatum</i>	China, Tibet, estuary eastern inflow Bangong Co	33.55763°	79.93183°	UGSB 12598		no sequence information	
CN24/4	<i>Pisidium zugmayeri</i>	China, Tibet, estuary eastern inflow Bangong Co	33.55763°	79.93183°	UGSB 12599		no sequence information	
CN25	<i>Musculium kashmirensis</i>	China, Tibet, death channel south of Bangong Co	33.23835°	79.78357°	UGSB 10444		no sequence information	
CN26/1	<i>Pisidium stewarti</i>	China, Tibet, eastern inflow Bangong Co	33.55812°	80.05582°	UGSB 12600		no sequence information	
CN26/2	<i>Pisidium zugmayeri</i>	China, Tibet, eastern inflow Bangong Co	33.55812°	80.05582°	UGSB 12601		no sequence information	
CN27	<i>Musculium kashmirensis</i>	China, Tibet, southern inflow Bangong Co	33.41452°	79.64413°	UGSB 10488		no sequence information	
DE01/1^	<i>Pisidium nitidum</i>	Germany, Mecklenburg-Vorpommern	53.58138°	10.95250°	UGSB 10561	KF483385	EU5559134	
DE02/1^	<i>Pisidium casertanum</i>	Germany, Brandenburg	52.24666°	14.45416°	UGSB 10562	KF483386	EU5559092	
DE03/1	<i>Pisidium globulare</i>	Germany, Sachsen	51.33000°	14.48250°	UGSB 10563	KF483423		KF483334
DE04/1	<i>Pisidium supinum</i>	Germany, Brandenburg	52.48727°	13.64439°	UGSB 10564	KF483388		
DE05/1	<i>Pisidium obtusale</i>	Germany, Thüringen	51.08027°	10.48500°	UGSB 10565	KF483387		KF483335

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Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen- Bank acc.#	16S Gen- Bank acc.#	28S Gen- Bank acc.#
GE01/1	<i>Pisidium personatum</i>	Georgia, Mzcheta-Mtianeti	42.07222°	44.95972°	UGSB 9045	KF483389	KF483288	KF483336
GE02/1	<i>Pisidium subtruncatum</i>	Georgia, Samegreto-Zemo Svaneti	42.23306°	41.84722°	UGSB 9047	KF483390	KF483287	KF483337
GE03/1	<i>Musculium lacustre</i>	Georgia, Gurien	42.04722°	41.76638°	UGSB 9048	KF483391	KF483289	
GR01/1	<i>Pisidium casertanum</i>	Greece, West Macedonia	40.90222°	21.82500°	UGSB 9075	KF483392	KF483290	KF483338
ID01/1	<i>Pisidium</i> sp.	Indonesia, Sulawesi	02.76075°	121.55860°	UGSB 10983		KF483291	KF483339
ID02/1	<i>Pisidium</i> cf. <i>dammermani</i>	Indonesia, Sulawesi	02.47667°	121.25977°	UGSB 10984	KF483393	KF483292	KF483340
IR01/1	<i>Pisidium casertanum</i>	Iran, Fars	29.57083°	51.94250°	UGSB 11142	KF483394	KF483293	KF483341
IT01/1	<i>Pisidium subtruncatum</i>	Italy, Caserta	41.09786°	14.33066°	UGSB 10566	KF483395	KF483294	
LS01/1	<i>Pisidium viridarium</i>	Lesotho, Thaba-Tseka	-29.51302°	28.52350°	UGSB 10567	KF483397	KF483296	
LS02/1	<i>Pisidium</i> cf. <i>casertanum</i>	Lesotho, Mokhotlong	-29.11645°	28.86351°	UGSB 10568	KF483396	KF483295	
MK01/1	<i>Pisidium henslowanum</i>	Macedonia, municipality of Resen	40.93665°	20.94414°	UGSB 10546	KF483398	KF483297	
MK02/1	<i>Pisidium maasseni</i>	Macedonia, municipality of Resen	40.87103°	20.98772°	UGSB 10549		KF483298	
MK03/1	<i>Pisidium</i> cf. <i>casertanum</i>	Macedonia, municipality of Struga	41.34999°	20.62245°	UGSB 10556	KF483399	KF483299	
MK04/1^	<i>Pisidium casertanum</i>	Macedonia, municipality of Struga	41.18526°	20.72862°	UGSB 10557	KF483400	EU559091	
MK05/1	<i>Pisidium personatum</i>	Macedonia, municipality of Resen	40.97319°	21.05432°	UGSB 10550	KF483401		
MK06/1^	<i>Pisidium subtruncatum</i> s. str.	Macedonia, municipality of Ohrid	40.91393°	20.74226°	UGSB 10559	KF483402	EU559153	
MK07/1	<i>Pisidium subtruncatum</i> <i>recalvum</i>	Macedonia, municipality of Ohrid	40.92063°	20.73580°	UGSB 10560		KF483300	
MK08/1	<i>Pisidium edlaueri</i>	Macedonia, municipality of Struga	41.09974°	20.63804°	UGSB 9058	KF483403	KF483301	KF483342
MK08/2	<i>Pisidium edlaueri</i>	Macedonia, municipality of Struga	41.09974°	20.63804°	UGSB 9059	KF483404	KF483302	
MW01/1	<i>Eupera</i> sp.	Malawi, Kassala	15.44374°	34.86392°	UGSB1224	KF483405	KF483303	
NA01/1	<i>Eupera</i> sp.	Namibia, Caprivi	-17.48629°	24.25615°	UGSB 5682		KF483304	

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Spec. Code	Taxon	Locality	Latitude	Longitude	Spec. voucher #	COI Gen- Bank acc.#	16S Gen- Bank acc.#	28S Gen- Bank acc.#
NO01/1	<i>Pisidium lilljeborgii</i>	Norway, Nordland	68.25166°	13.65750°	UGSB 10598	KF483406	KF483305	KF483343
NP01/1	<i>Pisidium kuiperi</i>	Nepal, Karnali	29.08333°	82.65000°	UGSB 10570	KF483407	KF483306	
NP02/1	<i>Pisidium atkinsonianum</i>	Nepal, Karnali	29.30000°	82.28333°	UGSB 10571	KF483408	KF483307	
NP03/1	<i>Musculium kashmirensis</i>	Nepal, Bagmati	27.59693°	85.37914°	UGSB 10573		KF483308	
NP03/2	<i>Pisidium annandalei</i>	Nepal, Bagmati	27.59693°	85.37914°	UGSB 9094	KF483409	KF483309	KF483330
NP03/3	<i>Pisidium kuiperi</i>	Nepal, Bagmati	27.59693°	85.37914°	UGSB 9097	KF483410	KF483310	KF483344
NP03/4	<i>Musculium indicum</i>	Nepal, Bagmati	27.59693°	85.37914°	UGSB 9098	KF483411	KF483311	KF483345
NP04/1	<i>Pisidium</i> sp.	Nepal, Mechi	27.51667°	87.70000°	UGSB 10577	KF483412	KF483312	KF483346
NP04/2	<i>Pisidium atkinsonianum</i>	Nepal, Mechi	27.51667°	87.70000°	UGSB 10594	KF483413	KF483313	
NP05/1	<i>Pisidium</i> sp.	Nepal, Mechi	27.48717°	87.74000°	UGSB 10583	KF483414	KF483314	
NP06/1	<i>Pisidium annandalei</i>	Nepal, Seti	29.47972°	81.12778°	UGSB 7004	KF483415	KF483315	
TR01/1	<i>Pisidium</i> cf. <i>nitidum</i>	Turkey, Isparta	37.88664°	30.85872°	UGSB 9064	KF483416	KF483317	KF483347
TR02/1	<i>Pisidium casertanum</i>	Turkey, Antalya	37.10960°	30.58551°	UGSB 9067	KF483418	KF483316	KF483348
TR02/2	<i>Pisidium personatum</i>	Turkey, Antalya	37.10960°	30.58551°	UGSB 9069	KF483417	KF483318	
UG01/1	<i>Sphaerium</i> sp.	Uganda, Mukono	0.22240°	32.63433°	UGSB 4856	KF483419	KF483319	KF483349
UG01/2	<i>Eupera ferruginea</i>	Uganda, Mukono	0.22240°	32.63433°	UGSB 4854	KF483420	KF483320	
UG02/1	<i>Eupera ferruginea</i>	Uganda, Buliisa	2.27527°	31.67599°	UGSB 5398	KF483421	KF483321	
VN01/1	<i>Pisidium nevillianum</i>	Vietnam, Ninh Binh	20.23361°	105.72194°	UGSB 10986		KF483322	KF483350
ZA01/1	<i>Pisidium costulosum</i>	South Africa, Mpumalanga	-27.01371°	29.87412°	UGSB 10599	KF483422	KF483323	

APPENDIX 2. List of additional 16S sequences obtained from GenBank including accession number, taxon, specimen code, locality information, and respective author(s).

16S Gen-Bank acc.#	Taxon	Spec. Code	Locality	Author(s)
AF152027	<i>Pisidium dubium</i>	US01/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152029	<i>Pisidium compressum</i>	US02/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152030	<i>Pisidium variabile</i>	US03/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152031	<i>Pisidium adamsi</i>	US04/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152032	<i>Pisidium sterkianum</i>	AR01/1*	Argentina, Buenos Aires	Cooley & Ó Foighil, 2000
AF152033	<i>Musculium securis</i>	US05/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152034	<i>Musculium argentinum</i>	AR01/2*	Argentina, Buenos Aires	Cooley & Ó Foighil, 2000
AF152035	<i>Musculium lacustre</i>	DE06/1*	Germany	Cooley & Ó Foighil, 2000
AF152036	<i>Musculium partumeium</i>	US06/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152037	<i>Sphaerium corneum</i>	DE07/1*	Germany	Cooley & Ó Foighil, 2000
AF152038	<i>Sphaerium rhomboideum</i>	US07/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152039	<i>Sphaerium fabale</i>	US08/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152040	<i>Sphaerium simile</i>	US09/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152045	<i>Sphaerium striatinum</i>	US10/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152046	<i>Sphaerium occidentale</i>	US11/1*	USA, Michigan	Cooley & Ó Foighil, 2000
AF152047	<i>Sphaerium novaezealandiae</i>	NZ01/1*	New Zealand	Cooley & Ó Foighil, 2000
AF152048	<i>Sphaerium tasmanicum</i>	AU01/1*	Australia, New South Wales	Cooley & Ó Foighil, 2000
AY093550	<i>Musculium japonicum</i>	JP01/1*	Japan, Ehime Prefecture	Lee & Ó Foighil, 2003
AY093552	<i>Musculium lacustre</i>	US12/1*	USA, Michigan	Lee & Ó Foighil, 2003
AY093553	<i>Musculium miyadaii</i>	RZ01/1*	Russia, Kuril Islands	Lee & Ó Foighil, 2003
AY093554	<i>Musculium transversum</i>	US13/1*	USA, Michigan	Lee & Ó Foighil, 2003
AY093557	<i>Pisidium casertanum</i>	DE08/1*	Germany, Ammerbuch	Lee & Ó Foighil, 2003
AY093559	<i>Pisidium casertanum</i>	US14/1*	USA, Michigan	Lee & Ó Foighil, 2003
AY093562	<i>Pisidium hallae</i>	N/A.1*	N/A	Lee & Ó Foighil, 2003
AY093565	<i>Pisidium nipponense</i>	JP02/1*	Japan, Gunma Prefecture	Lee & Ó Foighil, 2003
AY093566	<i>Pisidium nitidum</i>	N/A.2*	N/A	Lee & Ó Foighil, 2003
AY093569	<i>Pisidium supinum</i>	N/A.3*	N/A	Lee & Ó Foighil, 2003
AY093570	<i>Pisidium parvum</i>	JP01/2*	Japan, Ehime Prefecture	Lee & Ó Foighil, 2003
AY093571	<i>Pisidium japonicum</i>	JP03/1*	Japan, Nagano Prefecture	Lee & Ó Foighil, 2003
AY093573	<i>Sphaerium nucleus</i>	UA01/1*	Ukraine	Lee & Ó Foighil, 2003
AY792320	<i>Sphaerium corneum</i>	LT01/1*	Lithuania, Vilnius	Petkeviciute et al., 2006
AY957824	<i>Pisidium fallax</i>	US15/1*	USA, Colorado	Guralnick, 2005
AY957832	<i>Pisidium hallae</i>	US16/1*	USA	Guralnick, 2005
AY957836	<i>Pisidium ventricosum</i>	US17/1*	USA, Colorado	Guralnick, 2005
AY957855	<i>Pisidium milium</i>	US18/1*	USA, Colorado	Guralnick, 2005
AY957858	<i>Pisidium nitidum</i>	US19/1*	USA, Colorado	Guralnick, 2005
AY957880	<i>Pisidium ferrugineum</i>	US20/1*	USA	Guralnick, 2005
AY957884	<i>Pisidium ferrugineum</i>	US21/1*	USA	Guralnick, 2005

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16S Gen-Bank acc.#	Taxon	Spec. Code	Locality	Author(s)
DQ062627	<i>Pisidium moitesserianum</i>	N/A.4*	N/A	Steiner, 2006 (unpubl. data)
EU559087	<i>Pisidium amnicum</i>	AL02/1*	Albania, Tušemišta, Lake Ohrid	Schultheiss et al., 2008
EU559089	<i>Pisidium amnicum</i>	ME01/1*	Montenegro, Crnojevica River	Schultheiss et al., 2008
EU559090	<i>Pisidium annandalei</i>	IN01/1*	India, Pithoragarh	Schultheiss et al., 2008
EU559095	<i>Pisidium casertanum</i>	MK05/2*	Macedonia, Pretor, Lake Prespa	Schultheiss et al., 2008
EU559097	<i>Pisidium casertanum</i>	ES01/1*	Spain, Picos de Europa	Schultheiss et al., 2008
EU559104	<i>Pisidium clarkeanum</i>	NP07/1*	Nepal, Ghatgai w Sauraha	Schultheiss et al., 2008
EU559105	<i>Pisidium clarkeanum</i>	NP08/1*	Nepal, Haripur	Schultheiss et al., 2008
EU559110	<i>Pisidium edlaueri</i>	MK09/1*	Macedonia, Struga, Lake Ohrid	Schultheiss et al., 2008
EU559111	<i>Pisidium edlaueri</i>	MK10/1*	Macedonia, Ohrid town, Lake Ohrid	Schultheiss et al., 2008
EU559133	<i>Pisidium nevillianum</i>	NP09/1*	Nepal, Lamahi	Schultheiss et al., 2008
EU559147	<i>Pisidium pseudosphaerium</i>	DE03/2*	Germany, Uhyst	Schultheiss et al., 2008
EU559164	<i>Pisidium waldeni</i>	NO02/1*	Norway, Davggejavrre	Schultheiss et al., 2008

AN IMMUNOLABELLING TECHNIQUE TO TRACK SPERM FROM DIFFERENT MATES IN THE FEMALE REPRODUCTIVE ORGANS OF TERRESTRIAL GASTROPODS

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ABSTRACT

The mechanisms of sperm transfer, storage, utilization and digestion are crucial for understanding processes of postcopulatory sexual selection. Previous studies analysing postcopulatory processes have generally focused only on the ultimate outcome of the interactions between male and female sexual selection (paternity patterns). For a mechanistic understanding of the fate of received sperm and the involved patterns of postcopulatory sexual selection new techniques are required. Here we present an improved immunolabelling technique to track the fate of 5-bromo-2'-deoxyuridine (BrdU)-labelled sperm in the female reproductive organs of gastropods. The technique was tested in individuals of the simultaneously hermaphroditic land snail *Arianta arbustorum* (Linnaeus, 1758). We determined the percentage of labelled sperm in spermatophores delivered and assessed the reliability of detecting labelled sperm in the spermatheca (sperm storage organ) and bursa copulatrix (sperm digestion organ) of the recipients. In our tests, the proportion of sperm labelled among the sperm produced by an individual averaged 99.3%. Furthermore, labelled sperm could be consistently visualized in both the sperm storage and sperm digestion organ of all recipients examined. Combined with a traditional sperm staining technique, we showed that the BrdU-labelling technique allows us to distinguish between sperm from two males in the female reproductive tract of double-mated snails. In a controlled growth experiment, we found that repeated BrdU-application slightly retarded shell growth of juvenile snails, but did not influence their adult size, survival, number of sperm delivered and mating frequency. The technique presented could be adjusted to other gastropod species providing insight into mechanisms of sperm competition and cryptic female choice.

Key words: *Arianta arbustorum*, female reproductive organs, Gastropoda, immunolabelling, postcopulatory sexual selection, spermatheca, sperm digesting organ, sperm storage.

INTRODUCTION

Sexual selection continues after copulation in many animal species by way of sperm competition and female sperm manipulation (Birkhead & Møller, 1998). Sperm competition occurs when spermatozoa from different males compete in the reproductive tract of a female for the fertilization of her eggs (Parker, 1970). In a variety of species, females have a physiologically and morphologically complex reproductive system, which may enable them to influence or even control offspring paternity by postcopulatory sperm storage and selective sperm use (frequently summarized as cryptic female choice; Eberhard, 1996). Disentan-

gling patterns of sperm storage, sperm use and sperm digestion is the key to understand mechanisms of sperm competition and cryptic female choice (Birkhead & Møller, 1998). So far, most studies infer these patterns from paternity data, which only reveal the ultimate outcomes of the interactions between male and female reproductive processes (Schärer et al., 2007). With a mechanistic understanding of the fate of received sperm, and the involved patterns of postcopulatory sexual selection, we can better understand the evolution of male and female reproductive morphology and physiology.

Different approaches have been developed to track the donor's sperm in the recipient. Using radiolabelled nucleotides, which result in

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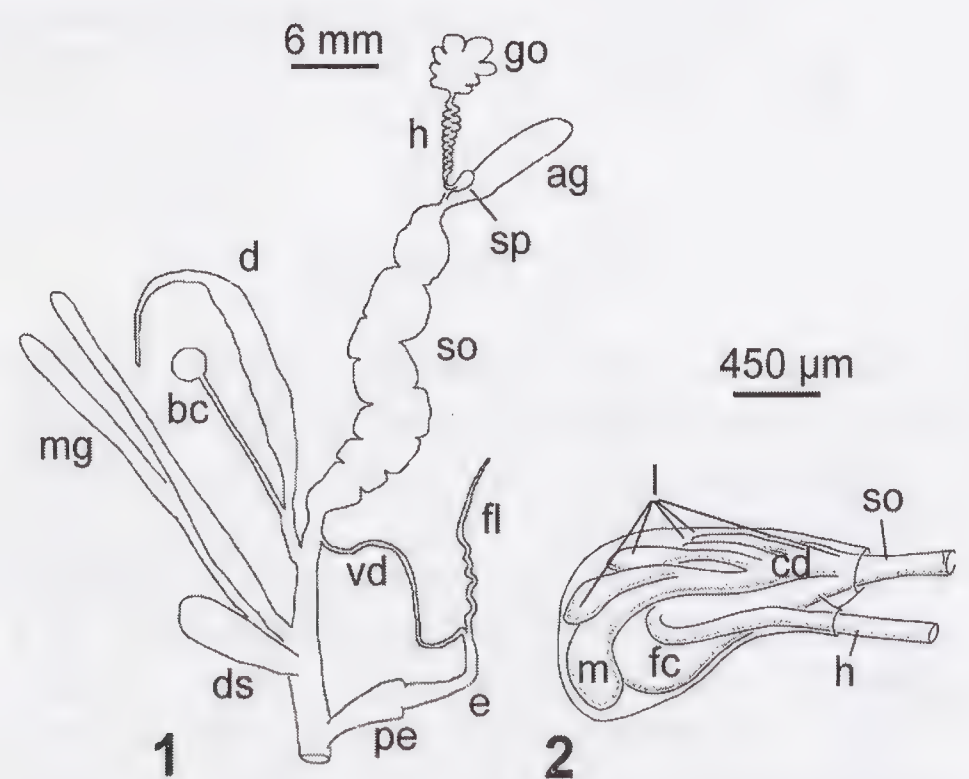
a specific labelling of the DNA of the donor's sperm, the position of sperm in the female tract can be located by scintillation counting or autoradiography (e.g., Beeman, 1970; Nollen, 1975; Bishop, 1996). However, this approach has several limitations due to radioactivity and laboratory facility requirements. Recently, insertions of genes for a green-fluorescent protein in the genome of *Drosophila melanogaster* have been used to distinguish sperm from different males (Civetta, 1999; Manier et al., 2010). The successful application of this method requires an extensive knowledge of the genome of the target species that is currently only available for a few species. Microsatellite competitive PCR is another technique for quantifying the relative number of sperm from different males stored in the spermatheca of multiple mated females (Bussi re et al., 2010; Hall et al., 2010). However, this technique is of limited use when postcopulatory female choice (sperm storage or digestion) is based on the genetic heterozygosity or complementarity of the mating males (e.g., Minoretta et al., 2011).

In recent years, immunocytochemical techniques have been developed to visualize particular types of cells. For example, 5-bromo-2'-deoxyuridine (hereafter called BrdU), a synthetic analogue of the nucleic acid component thymidine, is incorporated into newly synthesized nucleic acids by substituting for thymidine. BrdU can be visualized with immunocytochemical staining methods. This technique is commonly used for cancer cell detection in humans and in cell development research (Soames et al., 1994). In a few gastropod species, BrdU has been used to investigate brain cell development and the immune defense system (Zakharov et al., 1998; Gorbushin & Iakovleva, 2006). Sch rer et al. (2007) and Janicke & Sch rer (2009) used the BrdU-immunocytochemical approach to track sperm in the transparent flatworm *Macrostomum lignano*. Individuals of this free-living flatworm were labelled by incubation in BrdU-enriched artificial seawater. There was a considerable loss of flatworms in the staining process (36%; Sch rer et al. 2007), and no data on the percentage of labelled sperm have been reported. In the present study, we further developed the BrdU-labelling method to track sperm received in the female organs of single and double-mated individuals of the simultaneously hermaphroditic terrestrial pulmonate *Arianta arbustorum* (Linnaeus, 1758).

In stylommatophoran gastropods, the site of storage (= spermatheca) of received sperm

(allosperm) shows an enormous interspecific variability in morphology and number of tubules (reviewed in Baur, 2010). For example, *Oxychilus draparnaudi* (Beck 1837) and *Bradybaena fruticum* (M ller 1774) have a single spermathecal tubule. In *Succinea putris* (Linnaeus, 1758) two spermathecal tubules occur, and 34 tubules have been recorded in the spermatheca of *Drymaeus papyraceus* Mawe, 1823. There is also a considerable within-species variation in the number of spermathecal tubules (e.g., 3–5 in *Helix pomatia* Linnaeus, 1758; 4–19 in *Cornu aspersum* M ller, 1774; Evanno & Madec, 2007; Koentzopoulos & Staikou, 2007). As a consequence of the large intraspecific variation in the number of spermathecal tubules, different individuals have different possibilities to store allosperm from more than one mating partner. Mixing of sperm from different mates is more likely in a less structured spermatheca, whereas a large number of tubules potentially allows better separation of spermatozoa from different mates.

In stylommatophoran gastropods, the received sperm travel through the spermoviduct to the spermatheca (Figs. 1, 2), where a minor fraction is stored (Lind, 1973; Rogers & Chase, 2001). The majority of sperm, however, are directed to the sperm-digesting bursa copulatrix (Beese et al., 2006a; Fig. 1). In the spermath-



FIGS. 1, 2. Schematic representations of reproductive system of *A. arbustorum*. FIG. 1: Line drawing of reproductive system; FIG. 2: Line drawing of spermatheca; Abbreviations: ag albumen gland, bc bursa copulatrix, cd common duct, d diverticulum, ds dart sac, e epiphallus, fc fertilization chamber, fl flagellum, go gonad, h hermaphroditic duct, l lateral tubules, m main tubule, mg mucous glands, pe penis, sp spermatheca, so sperm oviduct, vd vas deferens.

eca, allosperm are stored for long periods (up to four years; Baur, 1998). Sperm survive best when attached to the walls of spermathecal tubuli (Chase & Darbyson, 2008).

The aim of our study was to further develop the BrdU-technique to distinguish between spermatozoa from two donors (one labelled, the other unlabelled) stored in the spermatheca of *A. arbustorum*. We injected BrdU into growing individuals of *A. arbustorum* and conducted several tests to assess the reliability of sperm labelling in the donor snail, to distinguish between labelled and unlabelled sperm and to demonstrate labelled sperm in different organs of the sperm-receiving mating partner. We also examined whether repeated BrdU-injections influence shell growth and adult size of snails. Finally, we present a first image showing the differential storage of sperm from two donors in the highly structured spermatheca of a double-mated individual of *A. arbustorum* and describe a method to quantify the relative amounts of sperm stored from both donors.

MATERIALS AND METHODS

Study Organism

Arianta arbustorum is common in moist habitats of northwestern and central Europe (Kerney & Cameron, 1979). The snail has determinate growth (shell width of adults 17–22 mm; Baur, 1984). Individuals become sexually mature at 2–4 years and adults live another 3–4 years (Baur & Raboud, 1988). Mating is random with respect to shell size, different degrees of relatedness and mating history (Baur, 1992; Baur & Baur, 1997; Kupfernagel & Baur, 2011). Copulation is reciprocal; both snails transfer simultaneously one spermatophore (Baur, 2007). The number of sperm delivered varies considerably (803,000–3,969,000), with an average of 2,185,000 (Baur et al., 1998). However, studies in other helicid snails revealed that only a minor fraction of sperm received are stored in the spermatheca (0.02–0.10%; Lind, 1973; Rogers & Chase, 2002). Snails need at least eight days to replenish their sperm reserves after a successful copulation (Locher & Baur, 1999). Sperm length varies from 878 to 939 μm in *A. arbustorum* (Fig. 3; Minoretti & Baur, 2006; Minoretti et al., 2013).

The sperm storage organ of *A. arbustorum* shows a considerable variation in the number of blind-ending spermathecal tubules (2–9). The tubules unite into a common duct that

opens into the fertilization chamber (Fig. 2; Baminger et al., 2000; Beese et al., 2009). Incoming sperm are not equally distributed among all spermathecal tubules, suggesting that sperm from different partners might be stored separately (Baur, 1994; Haase & Baur, 1995; Bojat & Haase, 2002). The musculature surrounding the spermathecal tubules is arranged in a complex three-dimensional network (Bojat et al., 2001a). If there were a selective activation of the muscles of each tubule (which has not yet been examined), this might allow the animal to selectively utilize sperm stored in single tubules and thus promote a selective fertilization of eggs (Baur, 2010).

In *A. arbustorum*, the dominant mode of reproduction is cross-fertilization. The snails mate repeatedly in the course of a reproductive season, and fertile sperm from different partners can be stored for more than one year in the sperm storage organ (Baur, 1988). There is a probability of 5–8% that a copulation will not lead to fertilization of eggs (no sperm or spermatophore transfer or transfer of infertile sperm; Chen & Baur, 1993). In the absence of potential mating partners, a low rate of self-fertilization can be observed both in breeding experiments and in natural populations (Chen, 1994; Kupfernagel et al., 2010).

Snail Sampling and Maintenance

Juvenile individuals of *A. arbustorum* (shell width: 8–12 mm) were collected in the sub-alpine forest near Gurnigelbad, Switzerland (46°45.5'N, 7°27.5'E, elevation 1,230 m a.s.l.) on 14 May 2009 and 30 April 2012, 2–4 weeks after hibernation. Snails were kept individually in transparent beakers (8 cm deep, 6.5 cm in diameter) lined with moist soil (approximately 4 cm) at 19°C with a light:dark cycle of 16:8 h.

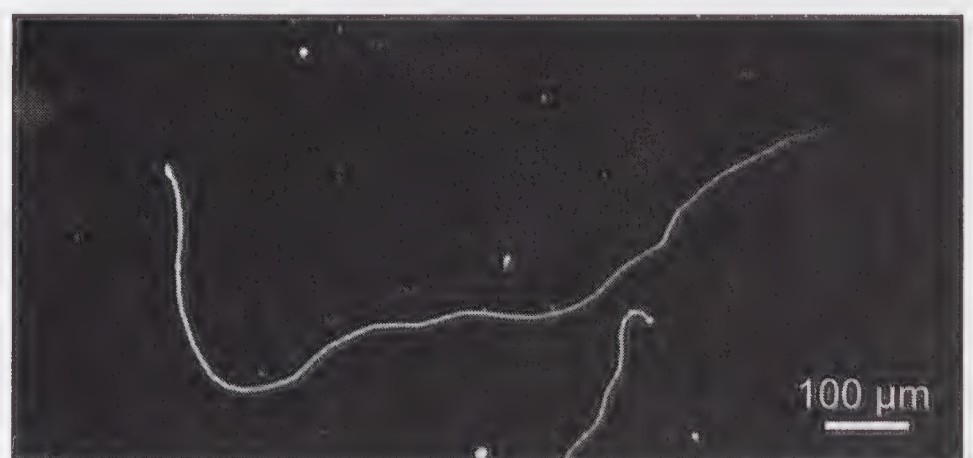


FIG. 3. Spermatozoon of *A. arbustorum* (unlabelled; microscopical phase contrast image). In this species, sperm measures 878–939 μm in length of which the head accounts for 15–25 μm (see Minoretti & Baur 2006).

Fresh lettuce was provided twice a week and at the same time the beakers were cleaned. In the wild, juvenile snails of the size range considered in this study may attain adulthood within 4–6 months and reproduce in the following year(s). Under the more favourable laboratory conditions, the juvenile snails reached sexual maturity, indicated by the formation of a reflected lip at the shell aperture, after 2–3 months. The snails were marked individually by writing numbers on their shells with a waterproof felt-tipped pen on a spot of correction fluid (Tipp-Ex). The animals showed no visible reaction to the marking procedure.

Sperm Labelling

Juvenile snails were randomly assigned either to a treatment group (hereafter called BrdU-labelled) or to a control group (hereafter called unlabelled). Individuals of the BrdU-labelled group received a BrdU injection (5-bromo-2'-deoxyuridine, Roth AG, Arlesheim, Switzerland; 1% in Ringer solution) every second week until they reached sexual maturity and this was continued afterwards, now as adults. Using a hypodermic syringe (needle size: 100 µm; U-100, BD Medical, Le Pont-de-Claix, France), 100 µl of solution was injected into the foot muscle, next to the retractory muscle and shell chamber. Absorption of BrdU due to skin immersion and/or injection has already been described in other invertebrate species (Zakharov et al., 1998; Gorbushin & Iakovleva, 2006; Schärer et al., 2007). BrdU is mainly incorporated into newly synthesized nucleic acid. Consequently, to ensure that all of the continuously produced sperm are labelled, BrdU-injections were repeated at intervals of two weeks over the course of the experiments.

BrdU Immunolabelling and DAPI Staining

To examine the fate of sperm in the female reproductive organs of the recipient, unlabelled snails were allowed to mate with BrdU-labelled individuals (for details see below). After successful copulation, recipients were killed by deep freezing (–80°C) and their female reproductive organs were dissected out and fixed for 2–4 h in paraformaldehyde (4%). Next, the reproductive organs were rinsed in distilled water (30–60 min), incubated in 50% ethanol (15 min) and finally stored in 70% ethanol. For immunolabelling and structural analyses, the

organs were embedded in paraplast, serially sectioned at 8 µm and positioned on Histo-Bond® microscope slides. One microscope slide carried 3–5 sectioned tissue slices. The slides were then deparaffinized in xylene and rehydrated through graded ethanol solutions to phosphate-buffered saline (PBS).

The tissue slides were treated with 0.5% trypsin (in EDTA; Invitrogen AG, Basel, Switzerland) for 20 min at 37°C, washed 5 min in PBS-T (PBS with 1% Triton X-100; Roth AG, Arlesheim, Switzerland), followed by a treatment with 2 N HCl for 30 min at room temperature to denature the DNA. After rinsing in PBS-T, enzyme activity and DNA denaturation were blocked with BSA-T (= PBS-T with 1% albumin fraction V; Roth AG, Arlesheim, Switzerland) for 15–30 min at 37°C. Tissue was incubated overnight at room temperature (alternatively for 4–5 h at 37°C) in a mouse monoclonal antibody directed against single-stranded DNA containing brome-associated-uridine (BU-33, diluted 1:50 in BSA-T; Sigma, Missouri, USA). After this, the slides were washed four times in PBS-T, followed by 15 min in goat serum (G9023; Sigma, Missouri, USA) at 37°C to block unspecific binding, followed by a 1 h incubation at 37°C with a goat-anti-mouse FITC-conjugated secondary antibody (1:100, F5897; Sigma, Missouri, USA) directed specifically against the BU-33 antibodies. With the incubation of the second antibody the cell nuclei marker DAPI (1:500; Roth AG, Arlesheim, Switzerland) was added for counterstaining. DAPI labels the DNA of all cells and can therefore be used to compare the number of BrdU-labelled sperm with the total number of sperm occurring in a reproductive organ. Following our protocol, DAPI-incorporation was inhibited in BrdU-labelled sperm. This fact can be used to recognize unlabelled sperm in recipient snails.

After final washing steps (three times with PBS-T), tissue slides were embedded in PBS-glycerol (ratio 1:1) with the anti-microbial substance Thimerosal (0.005%; Roth AG, Arlesheim, Switzerland) and coverslipped. After the staining procedure, the slides were stored at 4°C for up to seven days in darkness to prevent fading of fluorescence. For fluorescence visualization of BrdU-labelled sperm and cells, a microscope (Olympus AX-70) with magnifications ranging from 40 to 400 was used. For digital photography the imaging software SPOT version 4.6 (Diagnostic Instruments Inc., Michigan, 2006) was applied.

Proportion of Labelled Sperm

Two sets of mating trials were conducted to assess the percentage of labelled sperm among all sperm produced by individuals treated with BrdU. First, we assessed the proportion of labelled sperm delivered during the first copulation. BrdU-labelled individuals were allowed to mate with virgin unlabelled individuals (functioning as recipients) for the first time. In three recipients, the spermatophore received was dissected out immediately after copulation (described below). Second, we examined the percentage of labelled sperm produced by donor snails after the first copulation. Mated BrdU-labelled snails were allowed to replenish their autosperm reserves for 7–10 days. The snails received a further BrdU-injection one to two days after their first copulation. These snails were again paired with virgin unlabelled individuals serving as recipients and the spermatophores delivered by the BrdU-labelled snails were dissected out of the recipients. To examine whether labelled and control snails differ in number of sperm delivered during copulation, we also paired unlabelled individuals with unlabelled recipients ($n = 6$) and dissected out the spermatophores.

Sperm were extracted from the obtained spermatophores as described in Locher & Baur (1997). The sperm mass delivered by each BrdU-labelled individual was divided into two equal aliquots. The first was used for immunostaining. Sperm were stored at -20°C until preparation for immunostaining. From the sperm solution, 2 μl were loaded on HistoBond® microscope slides (Paul Marienfeld GmbH & Co KG, Lauda-Königshofen, Germany) and air-dried for immunostaining. After immunostaining, we counted the number of labelled (BrdU with conjugated fluorescence marker FITC) and unlabelled (DNA with fluorescence marker DAPI) sperm on images of four randomly chosen slide sections of each spermatophore. For sperm counting on the digital photographs, the open source programme ImageJ version 1.44 (<http://rsbweb.nih.gov/ij/>) was used. This software facilitates manual counting and particle analysis from images. Based on the counts of labelled and unlabelled sperm, the percentage of labelled sperm delivered in each spermatophore was calculated. The repeatability of this technique was assessed by using multiple measurements ($n = 4$) in randomly-chosen sections ($n = 3$) on photographs following Lessells & Boag (1987). The second aliquot was used for estimating the total number of sperm delivered with the spermatophore, following Locher & Baur (1997).

To examine whether sperm from two donors (one BrdU-labelled and the other unlabelled) can be distinguished, we compared the immunostaining of BrdU-labelled sperm and unlabelled sperm in a 1:1 mixture of sperm (each 1 μl) from both donors on microscope slides.

BrdU-Labelled and Unlabelled Sperm in the Female Organs of the Recipient

Mating trials were conducted to examine the reliability of immunostaining of labelled sperm in the female organs of unlabelled sperm receivers. The spermatheca and bursa copulatrix of six unlabelled individuals that mated with a BrdU-labelled snail were dissected out at 48 h after copulation. This period of time allows sperm to leave the spermatophore and to reach the sperm storage organ. The remaining sperm are transported into the bursa copulatrix. None of the snails had laid eggs within 48 h after copulation. In three pairs, the BrdU-labelled partner mated for the first time, in the other three pairs for the second time.

Spermatheca and bursa copulatrix were embedded in paraplast, serially cross-sectioned at 8 μm and labelled with FITC and DAPI. By combining information from 2–3 successive tissue slices, it is possible to estimate the number of sperm stored (sperm heads measure 15–25 μm ; Minoretti & Baur, 2006). However, it is extremely labour-consuming to count all the sperm stored in the organ. We therefore decided to quantify results as the percentage of labelled sperm in relation to all sperm in the organ (see below). To check for the occurrence of auto-fluorescence, the sperm, spermatheca and bursa copulatrix of two unlabelled snails that copulated with an unlabelled partner were investigated as described above.

Finally, to visualize the pattern of sperm storage in the spermatheca of double-mated *A. arbustorum*, virgin unlabelled individuals were allowed to copulate with two partners, first with an unlabelled snail and then with a BrdU-treated snail. The recipients were killed by freezing (-80°C) 48 h after copulation. The spermatheca were cross-sectioned at 8 μm and labelled with FITC and DAPI as described above.

Quantification of BrdU-Labelled and Unlabelled Sperm in the Spermatheca

To obtain a quantitative estimate of the relative amount of sperm stored from each of the two donors in the spermatheca of a double-mated individual of *A. arbustorum*, the areas covered

TABLE 1. Percentage of BrdU-labelled sperm and total number of sperm in spermatophores delivered by BrdU-treated individuals of *A. arbustorum* during the first and second copulation.

Snail ID	First/second copulation*	Labelled sperm (%) Mean (range)†	Total number of sperm delivered
L1	first	98.3 (96.7–100.0)	1.25 x 10 ⁶
L16.1	first	99.1 (97.9–100.0)	1.00 x 10 ⁶
L11	second	99.4 (98.5–100.0)	1.69 x 10 ⁶
L16.2	second	99.9 (99.4–100.0)	0.96 x 10 ⁶
L19	second	99.7 (99.1–100.0)	1.51 x 10 ⁶

* First: sperm were produced during growth before the first copulation; second: sperm were also produced between the two copulations.
† Labelled sperm were counted in each of three randomly chosen sections (each containing 29–488 immunostained sperm) from four different images per spermatophore.

by either type of sperm (BrdU-labelled and unlabelled) in the spermathecal tubules were assessed. Every tissue slice of the cross-sectioned spermatheca of the recipient was photographed and inspected for sperm. Only photographs with visible sperm heads were selected (= informative slices). The areas covered by sperm heads of either type on a slice were determined by counting the number of pixels for each sperm head type using Adobe Photoshop CS3 (Adobe Systems Inc., USA, 2007). To avoid counting the same sperm head twice in successive photographs, every fourth slice was examined. This necessary restriction resulted in 10 and 23 informative slices obtained from the spermatheca of two double-mated individuals. The number of informative slices varies among individuals because of size differences in the sperm storage organ and because the orientation of the spermatheca cannot be controlled before it is embedded in paraplast. For the estimate of the percentages of sperm stored from both donors, the sum of pixels of 10 slices per spermathecae was considered. Reducing the number of informative slices from 23 to 10 did not substantially change the percentages of sperm stored from either donor.

Effect of BrdU on Snail Growth and Adult Size

To examine whether repeated BrdU-injections influence shell growth, adult size and survival of *A. arbustorum*, the shell width of BrdU-labelled (n = 18) and control snails (n = 17) were measured three times to the nearest 0.1 mm using vernier calipers (immediately after sampling, after 42 and 134 days in summer 2012). Shell

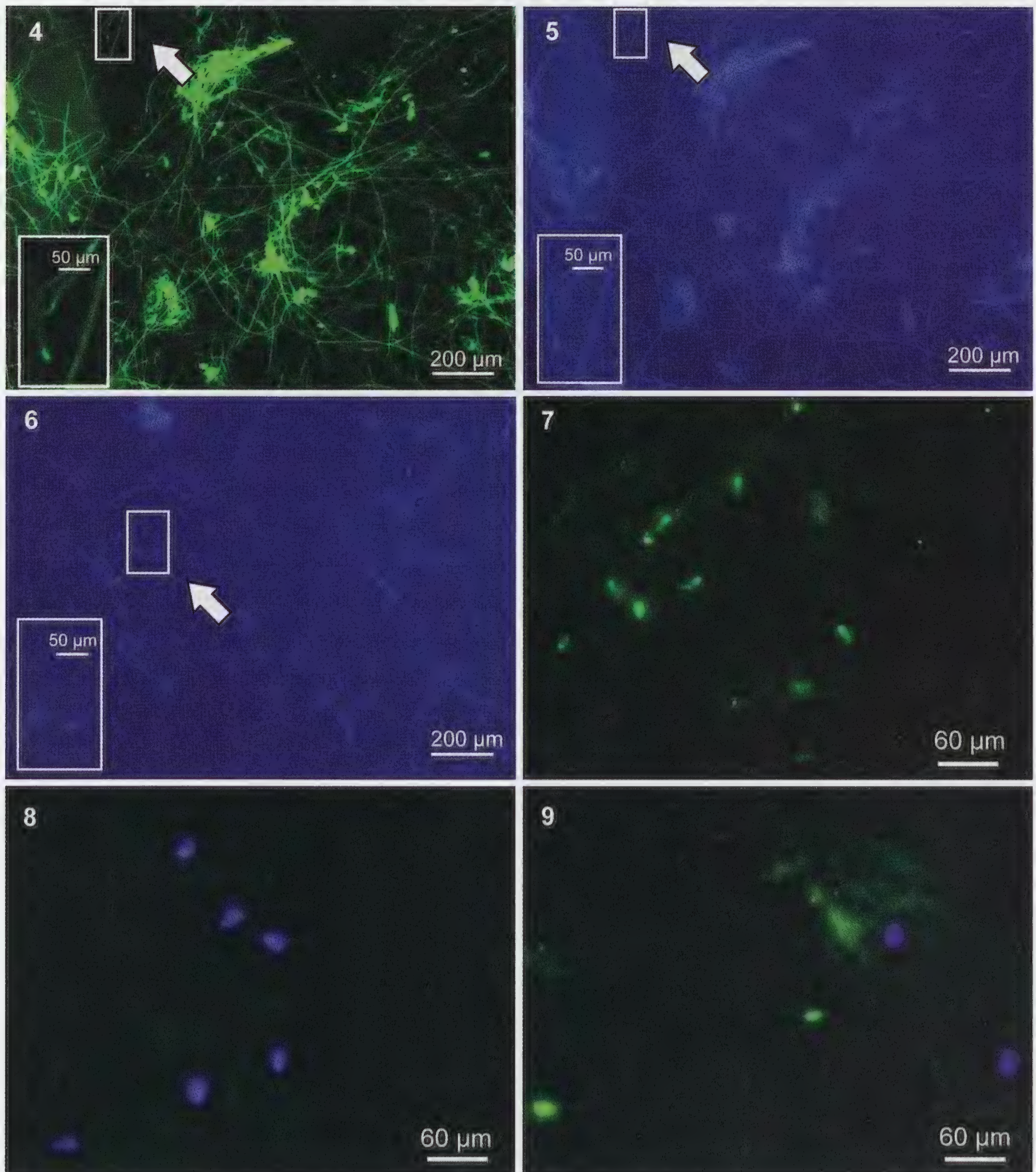
growth was expressed as growth rate (shell width increase per day in mm). Snail maintenance was as described above. Labelled snails received 10 BrdU-injections (each 100 µl of 1% solution) within 146 days (the time elapsed between two injections averaged 16.2 days). At the end of the experiment (after 160 days), the adult shell width of all individuals that had completed shell growth was measured and the number of surviving snails was determined for both groups. We also recorded the mating success of snails that reached sexual maturity.

All statistical analyses were conducted using PASW® statistics 19.0 core system (SPSS Inc., 2010).

RESULTS

Proportion of BrdU-Labelled Sperm

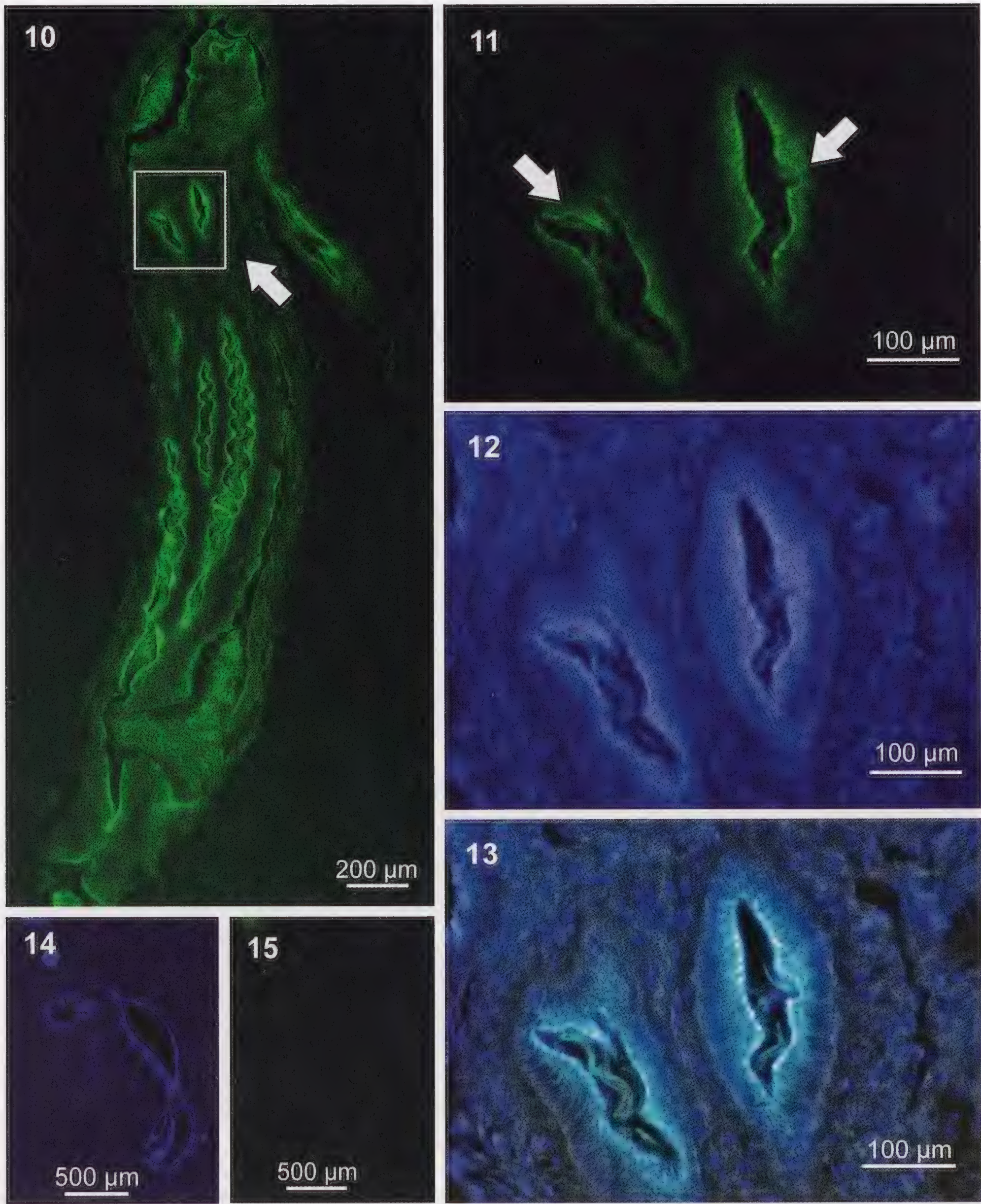
The spermatophore was obtained from two of three BrdU-treated snails that copulated for the first time and from all three snails that copulated for the second time (Table 1). One BrdU-labelled snail did not deliver a spermatophore. The percentage of labelled sperm among all sperm delivered in spermatophores of BrdU-treated snails averaged 99.3% (range: 98.3–99.9%; Table 1). The percentage of labelled sperm did not differ between snails that mated for the first time and those that mated for the second time (Mann-Whitney U-test: z = 1.70, n = 5, p = 0.21). The repeatability of the sperm counting method was 99.8% (F_{2,11} = 1502.3, p < 0.001).



FIGS. 4–9. BrdU-labelled and DAPI-stained sperm. Arrows indicate the location of one section, which is presented with higher magnification in the lower left corner of the image. FIG. 4: BrdU-labelled sperm visualized with FITC filter; FIG. 5: BrdU-labelled sperm (identical to Fig. 4) visualized with DAPI filter; FIG. 6: Unlabelled sperm visualized with DAPI filter; FIGS. 7–9: FITC and DAPI filter images are overlaid; FIG. 7: BrdU-labelled sperm; FIG. 8: Unlabelled sperm; FIG. 9: Sperm mixture (1:1) from a BrdU-labelled and an unlabelled sperm donor. Two BrdU-labelled and two unlabelled sperm heads are visible.

The total number of sperm delivered in spermatophores of BrdU-labelled individuals averaged 1.28×10^6 (range: 0.96 – 1.69×10^6 ; $n = 5$; Table 1). In unlabelled control individuals, the total number of sperm delivered aver-

aged 1.13×10^6 (range: 0.94 – 1.48×10^6 ; $n = 6$). There was no significant difference in the number of sperm delivered between labelled and unlabelled individuals ($t = 1.00$, $df = 9$, $p = 0.29$). These results indicate that both the label-



FIGS. 10–15. BrdU-labelling and DAPI-staining of spermatheca of single mated snails. FIG. 10: Cross section of spermatheca storing BrdU-labelled sperm emitting FITC fluorescence. Boxed area marked by the arrow indicates the area enlarged in FIGS. 11–13; FIG. 11: Spermathecal tubuli visualized with FITC filter. Arrows indicate BrdU-labelled sperm attached to the walls of the tubuli; FIG. 12: Spermathecal tubuli (identical to FIG. 11) visualized with DAPI filter; FIG. 13: Overlay image of FIGS. 11, 12; FIG. 14: Spermatheca storing unlabelled sperm visualized with DAPI-filter; FIG. 15: negative control: spermatheca storing unlabelled sperm visualized with FITC filter; no autofluorescence was visible.

ling method and the timing of BrdU-application are appropriate to achieve a high rate of sperm labelling without compromising sperm production (Figs. 4–6).

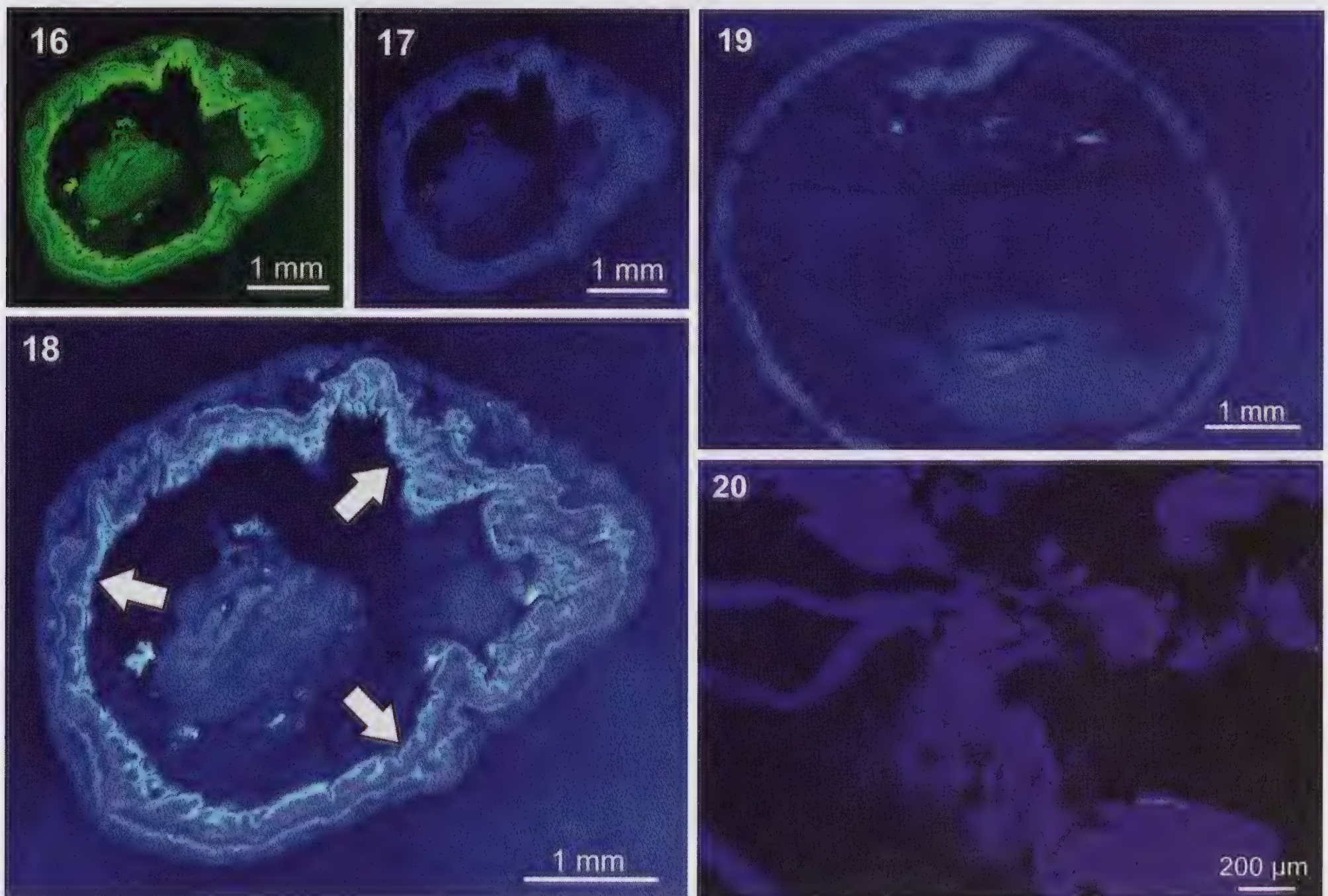
Sperm of a BrdU-labelled partner can be distinguished from sperm of an unlabelled partner (Figs. 7, 8). Overlaying BrdU/FITC- and DAPI-images allows for a count of the number of sperm heads of both types on single slides (Fig. 9). Based on a series of images the relative frequencies of sperm from either donor can be estimated (see below).

BrdU-Labelled and Unlabelled Sperm in the Female Organs of the Recipient

The spermatheca and bursa copulatrix of six unlabelled virgin snails that copulated with BrdU-treated individuals were checked for labelled sperm. All of the examined recipients stored BrdU-labelled sperm in the spermatheca

(Figs. 10–13). In control snails that mated with unlabelled individuals, sperm received could be visualized when stained with DAPI (Fig. 14), but not when stained with BrdU/FITC (Fig. 15), indicating no signs of auto-fluorescence. All snails that copulated with a BrdU-labelled partner had partly digested labelled sperm in the bursa copulatrix (Figs. 16–18). Similarly, control snails that copulated with unlabelled individuals showed partly digested unlabelled sperm in their bursa copulatrix (Figs. 19, 20). These results demonstrate that BrdU-labelled sperm entered the female reproductive organs of the recipients.

Figures 21–24 show a cross section of spermathecal tubules of a double-mated snail. The first mate was an unlabelled snail and the second one a BrdU-labelled individual. The time elapsed between the two copulations was 24 days. The overlay images (Figs. 23, 24) from BrdU/FITC immunolabelling and DAPI stain-



FIGS. 16–20. BrdU immunolabelling and DAPI-staining of bursa copulatrix. FIG. 16: Bursa copulatrix visualized with FITC filter, showing the presence of BrdU-labelled sperm; FIG. 17: Bursa copulatrix (identical to FIG. 16) visualized with DAPI filter; FIG. 18: Overlay image of FIGS. 16 & 17. Arrows indicate areas with sperm in the process of digestion; FIG. 19: Overlay image of FITC and DAPI filters of bursa copulatrix that only contains unlabelled sperm; FIG. 20: Overlay image of FITC and DAPI filters, showing enlarged section of bursa copulatrix that only contains unlabelled sperm.

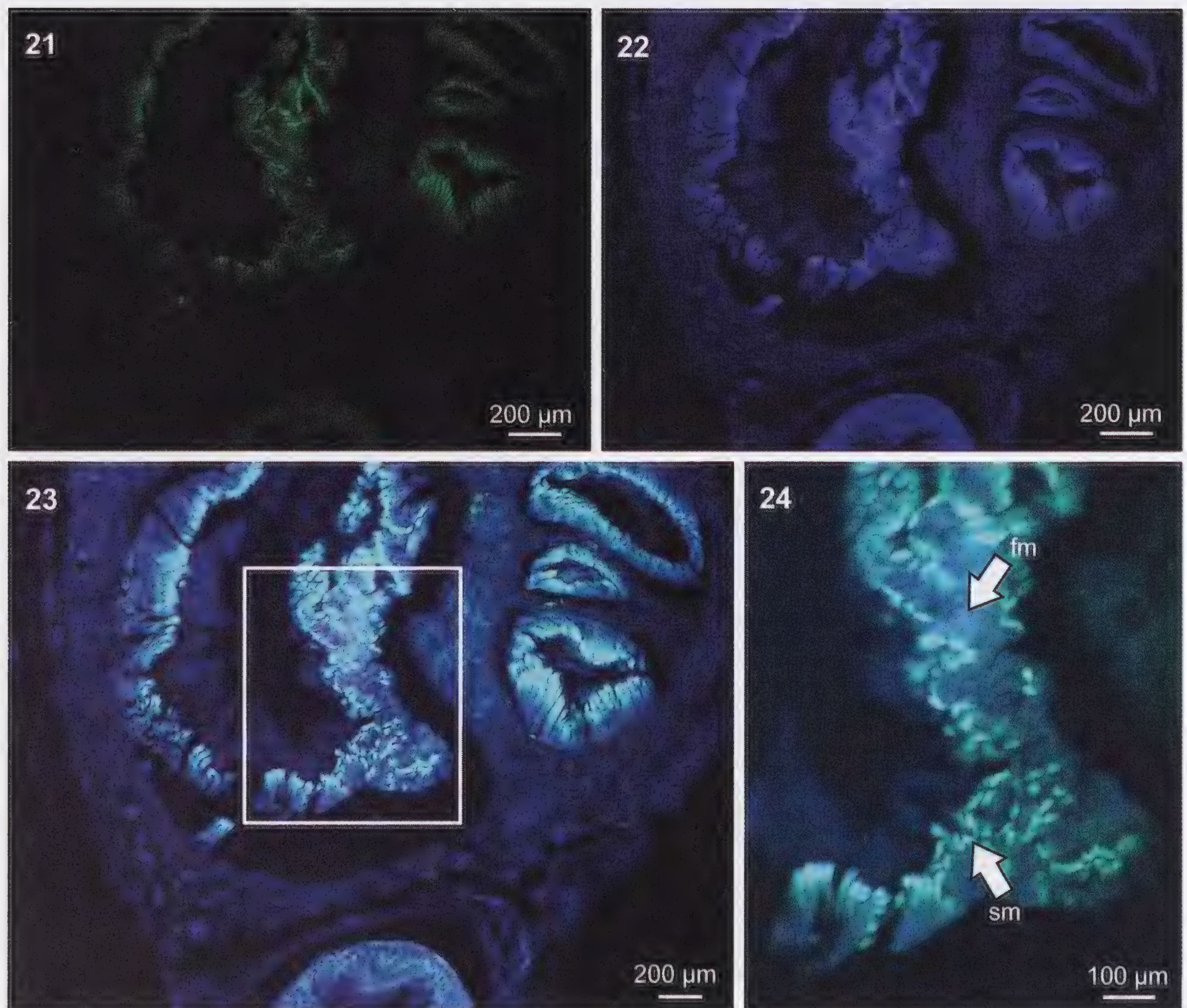
ing allow sperm from the two partners to be distinguished. Figure 23 shows a biased sperm storage. The majority of sperm stored in the main tubule were from the first mating partner. These sperm were attached to the wall of the tubule. Sperm from the second mating partner filled unoccupied parts of the spermathecal wall of the main tubule and three lateral tubules at the right upper corner of the image.

In the slice shown in Figure 23, 63% of the sperm stored were from the first mate (unlabelled sperm) and 37% from the second mate (BrdU-labelled sperm). Considering the entire spermatheca, 45% of the sperm stored were from the first mate and 55% from the second

mate (estimate based on ten slices; see Methods). The spermatheca of another double-mated snail contained 26% of the sperm from the first mate and 74% from the second mate (estimate based on ten slices).

Effect of BrdU on Snail Growth and Adult Size

Juvenile snails of the BrdU-labelled group did not differ in shell width from individuals of the control group at the beginning of the experiment (mean $\pm$ SE: 12.0 $\pm$ 0.2 mm vs. 12.1 $\pm$ 0.3 mm; $t = 0.32$, $df = 33$, $p = 0.75$). BrdU-injection seems to retard shell growth. In the first 42 days of the experiment (after three



FIGS. 21–24. BrdU-immunolabelled and DAPI-stained spermatheca of a double-mated individual. FIG. 21: Spermatheca showing multiple tubuli visualized with FITC filter; FIG. 22: Spermathecal tubuli (identical to FIG. 21) visualized with DAPI filter; FIG. 23: Overlay image of FIGS. 21 & 22. Boxed area indicates magnification shown in FIG. 24; FIG. 24: Section of main tubule showing BrdU-labelled and unlabelled sperm from two different donors. Abbreviations: *fm* first mate (with unlabelled sperm), *sm* second mate (with BrdU-labelled sperm).

injections), labelled snails had a reduced shell growth rate compared to control snails (0.073 ± 0.006 mmday⁻¹ vs. 0.098 ± 0.006 mmday⁻¹; $t = 2.90$, $df = 33$, $p = 0.0067$). The difference in growth rate became smaller but remained significant when a period of 134 days (after eight injections) was considered (0.028 ± 0.002 mmday⁻¹ vs. 0.034 ± 0.002 mmday⁻¹; $t = 2.34$, $df = 33$, $p = 0.0257$). However, long-term application of BrdU did not affect adult shell size. Juvenile snails randomly assigned either to the BrdU-treated group or the control group did not differ in adult size attained during the experiment (labelled vs. unlabelled: 17.1 ± 0.4 mm vs. 17.5 ± 0.3 mm; $t = 0.79$, $df = 13$, $p = 0.44$). In each group, one snail died during the experiment, suggesting that BrdU-injection did not increase mortality in *A. arbustorum* (survival of labelled vs. unlabelled: 94.4% vs. 94.1%; $X^2 = 0.002$, $df = 1$, $p = 0.96$).

Five BrdU-treated and nine control snails reached sexual maturity within 100 days. These snails were used in mating trials. Three of the five BrdU-treated snails mated (60.0%) as did six of the nine control snails (66.7%), indicating that there is no difference in mating propensity between the two groups of snails ($X^2 = 0.062$, $df = 1$, $p = 0.80$).

DISCUSSION

We presented a sperm-labelling technique that allows, in combination with a traditional staining method, the tracking of sperm from two donors (one with labelled sperm, the other with unlabelled sperm) in the reproductive organs of double-mated recipients in hermaphroditic gastropods. A series of tests demonstrate a high efficiency of the sperm-labelling procedure and show that labelled sperm can be visualized both in the sperm storage and sperm digesting organs of the recipient with a high reliability. This immunocytochemical approach opens new ways to investigate mechanisms and processes of postcopulatory sexual selection, including sperm transfer and both selective sperm storage and sperm utilization.

In the past, studies dealing with sperm distribution in the reproductive organs of the recipient utilising traditional histological staining methods could not distinguish between sperm from different mates. Moreover, sperm of radiolabelled males could not be precisely localized in the female reproductive tract (Bishop & Sommerfeldt, 1996). Most recently, insertions of fluorescence

genes were used to distinguish sperm of different males in the female tract in *D. melanogaster* (by Manier et al., 2010). However, such transgenic transformations require a detailed knowledge of the species genome for a successful application, which is so far available only for a few species. There is evidence that the degree of genetic heterozygosity of an individual influences its mating activity and reproductive success in *A. arbustorum* (by Minorette et al., 2011). If postcopulatory female choice (sperm storage or digestion) is based on the genetic heterozygosity or complementarity of mates, then competitive PCR is of limited use. In contrast, the improved immunostaining technique presented here may allow unbiased detection of distinctively labelled sperm in the female tract in a variety of gastropod species. Combined with traditional sperm staining methods, the immunostaining technique allows to distinguish between sperm from two males. It could even be further developed by including a second sperm labelling substance (Miller et al., 1991; Schärer et al., 2007). Furthermore, it could be adjusted to other invertebrate species.

In studies of postcopulatory selection, the application of BrdU should be well timed because the synthetic analogue is only incorporated into newly synthesized nucleic acids. To achieve a high rate of sperm labelling success a repeated or continuous application during growth and developmental of testes or ovotestis of the individuals as well as during sperm production is required. In a series of tests we could demonstrate a high rate of BrdU-labelling in sperm of *A. arbustorum*.

Long-term application of BrdU increases the risk of cell changes which may lead to cell death (Caldwell et al., 2005). Application of BrdU may also affect growth and reproduction. We found that repeated BrdU-injections retarded the shell growth of juvenile *A. arbustorum*, but did not influence the adult shell size attained and the number of sperm delivered in spermatophores. Furthermore, we did not record increased mortality or a decreased mating frequency in BrdU-treated individuals compared with control snails. Moreover, the distribution of BrdU-labelled sperm found in the sperm storage organ of the recipients did not differ from that of unlabelled sperm stained with traditional histological methods reported in previous studies (Baminger & Haase, 1999; Bojat et al., 2001b). This indicates that BrdU-labelling has some effects on the physiology of snails, but does not influence the male reproductive behaviour and sperm number in *A. arbustorum*. Thus, BrdU-

labelling may be a useful tool to study the fate of sperm in the female reproductive organs of the recipient. Combined with DAPI staining, immunostaining of BrdU-labelled sperm allows the investigation of sperm distribution in double-mated snails, i.e. BrdU-labelled vs. unlabelled sperm in the recipient.

We also presented a method to estimate the relative amount of sperm stored from each of the two donors in the spermatheca of the recipient. This estimate allows a comparison between the proportion of sperm stored and the percent of paternity (P_2 -value) in previously produced egg batches. Furthermore, this method allows estimates of the percentage of sperm stored in the main tubule and in each lateral tubule and thus to study the mechanism of sperm storage in double-mated snails.

We also showed that the sperm-labelling technique could be used to investigate the mechanisms of sperm digestion which might allow to follow digested particles originating from the labelled sperm in the recipient's tissue (e.g., to examine whether nucleic acids of digested sperm and spermatophores are incorporated in newly produced eggs). However, it should be kept in mind that the technique presented is limited to a snapshot in time, because of the tissue fixation to achieve fluorescence detection of labelled sperm. Consequently, dynamic processes in the sperm storage organ cannot be followed with this labelling technique.

In summary, the use of BrdU for sperm labelling has many applications in *A. arbustorum* and it could be adjusted to a variety of other gastropod and invertebrate species. With this technique, the fate of sperm can be followed in the female reproductive tract after copulation. In double-mated individuals, the sperm-labelling technique can give insight into mechanisms of postcopulatory sexual selection (Beese et al., 2006b). This technique may improve our understanding of the evolution of male and female morphology and physiology and of postcopulatory selection in general in hermaphroditic and gonochoristic species.

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COMPARATIVE MORPHOLOGY AND REDESCRIPTION OF THREE SPECIES OF *CALLIOSTOMA* (GASTROPODA, TROCHOIDEA) FROM BRAZILIAN COAST

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ABSTRACT

The morphology of *Calliostoma adpersum*, *C. depictum* and *C. hassler*, from the Brazilian coast, were studied in detail. Their anatomy has particularities that may be unique to the genus, such as: presence of an ampulla in the female; reduction or loss of the cephalic lappets; presence of a pseudoproboscis; occurrence of the intestinal loop outside the head-foot haemocoel. At species level, we conclude that *C. vinosum* is a synonym of *C. hassler*, because specimens identifiable as both species occur within a single ontogenetic series. *Calliostoma hassler* can be distinguished by: lack of the left dorsal esophageal fold, absence of a gastric spiral caecum and presence of an intestine with two wide loops. Based on anatomical evidence, we concluded that *C. depictum* is not a synonym of *C. adpersum*, differing by strongly convex whorls and a distinct suture; a marked groove near the columella; an odontophore partially covered by the mid-esophagus; absence of the pair of odontophore muscles m8a; a ventral esophageal fold of the same size as the dorsal folds; and a reduced spiral caecum with 0.5 whorl.

Key words: *Calliostoma*, comparative anatomy, Vetigastropoda, Calliostomatidae, Brazilian coast.

INTRODUCTION

The Calliostomatidae is a diverse family with a worldwide distribution and more than 250 recently described species (Marshall, 1995a). They are carnivorous, inhabit variable substrata normally associated with sessile invertebrates (Perron & Turner, 1978; Bryan et al., 1997; Hickman & McLean, 1990; Dornellas & Simone, 2011). Some recent molecular studies of Vetigastropod indicate Calliostomatidae to be closely related to Trochidae (Kano, 2008; Williams et al., 2008, 2010). In addition, the internal relationships of *Calliostoma* Swainson, 1840, is not well established and the taxonomic studies about the calliostomatid genera are restricted to some geographical regions (Clench & Turner, 1960; Marshall, 1995a, b).

The genus *Calliostoma* – type species, *Calliostoma conulum* (Linnaeus, 1781) – includes 95 species from the western Atlantic (Clench & Turner, 1960; Quinn, 1992). Previous investigations into the systematics of the genus have only used radulae, jaw and shell features (Ikebe, 1942; Clench & Turner, 1960; Quinn, 1992; Marshall, 1995a, b). The mainly characteristics

of *Calliostoma* are the presence of ampulla in females, which consist in a modification of the right kidney nephrostome; a pseudoproboscis; four pairs of epipodial tentacles; and a reduction or loss of the cephalic lappets. The radula in the subfamily Calliostomatinae has a low, reduced field of thin, highly flexible teeth with fine, serrate cusps. Calliostomatinae possess a stout, enlarged innermost marginal tooth with a heavy base and coarsely serrate hooked cusp. The serrate marginal teeth have a broad range of morphologies (Hickman & McLean, 1990).

Despite previous work on the genus, the internal anatomy of *Calliostoma* remains poorly understood and only few works described anatomical features (Randles, 1905; Fretter & Graham, 1962; Sá & Coelho, 1986; Dornellas, 2012). The relationships among calliostomatid species have proved difficult to understand, because the radular morphology and the external anatomy appear to be highly conservative within major groups. Traditional features (e.g., shell morphology) are not suitable for defining genera or subgenera, as a bewildering mosaic of intermediate character states exists (Marshall, 1995a).

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We aim to examine the external and internal anatomy of four species within the genus: *Calliostoma adpersum* (Philippi, 1845), *C. depictum* Dall, 1927, *C. hassler* Clench & Aguayo, 1939, and *C. vinosum* Quinn, 1992. In addition to the increase of specific definition of these species, which possess some taxonomical problems, this paper has also the intention of furnishing data for a future revaluation of the higher taxa, such as genus and family levels. Additionally, the proposed characters of the genus are investigated and updated and a new set of features to define the *Calliostoma* species is erected.

MATERIAL AND METHODS

Specimens were preserved in 70% alcohol extracted from their shells and dissected in a stereomicroscope with the specimen immersed in fixative. All drawings were made with a camera lucida. SEM examination of radulae, jaws and protoconch were made in the Laboratório de Microscopia Eletrônica do Museu de Zoologia da Universidade de São Paulo. The histological analysis were made in the Instituto Butantan. The specimens *in vivo* were analyzed and photographed under stereomicroscope without being extracted them from their shells. The terminology of the odontophore muscles follows Simone (2011).

Anatomical abbreviations: aa, anterior aorta; ab, bulbus aortae; af, afferent gill vessel; an, anus; ap, ampulla or urogenital papilla; bc, buccal commissure; bg, buccal ganglion; bl, buccolabial connective; bn, buccal nerve; cb, sub-esophageal connective; cc, cerebral commissure; ce, cerebral ganglion; cm, columellar muscle; cp, cerebropleural connective; cv, ctenidial vein or efferent gill vessel; cs, supra-esophageal connective; df, dorsal fold; dg, digestive gland; ef, esophageal folds; eg, esophageal gland or crop; el, esophageal papillae; em, mature spermatocyte; ep, epipodium; es, esophagus; et, epipodial tentacle; ev, esophageal valve; fs, foot sole (mesopodium); ft, foot; gi, gill; go, gonad; gs, gastric shield; hg, hypobranchial gland; il, intestinal loop; in, intestine; jw, jaws; la, left auricle; lc, spiral caecum; lf, left nephrostome; lg, labial ganglion; ln, neck lobe; lv, labial nerve; m1–m12, odontophore muscles; ma, mantle; mb, mantle border; mc, mouth sphincter; mj, jaw and peribuccal muscles; mo, mouth; mp, pericardium membrane; mt, transversal muscle; ng, nephridial gland; oa, opercular pad; oc, anterior odontophore cartilage;

od, odontophore; ol, out lip; om, ommatophore; on, optic nerve; oo, oogonia; op, operculum; os, osphradium; ov, oocyte; oy, mature oocyte; pa, papillae; pb, pseudoproboscis; pc, pericardium; pd, pedal cord; pe, pedal ganglion; pf, anterior furrow of pedal glands; pl, pleural ganglion; pm, cerebropedal connective; po, posterior odontophore cartilage; prs, posterior end of radular sac; ps, papillary sac; ra, radula; re, rectum; rk, right kidney; rs, radular sac; ru, right auricle; rv, right pallial vein; sa, posterior aorta; sc, subradular cartilage; sg, skeleton of gill; sm, subradular membrane; sn, snout; st, stomach; ta, statocyst; te, cephalic tentacle; ti, typhlossole; tn, tentacular nerve; to, tissue on radula; tr, trabeculae; um, suspensory membrane; ve, ventricle; vf, ventral fold; vt, transverse pallial vessel.

Institutional abbreviations: FSBC I, Invertebrate Collection, Florida Marine Research Institute, St. Petersburg, Florida; MCZ, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts; MfN, Museum für Naturkunde der Humboldt-Universität zu Berlin; MORG, Museu Oceanográfico Rio Grande, NHM, Natural History Museum, London; NMNH/USNM, National Museum of Natural History, Smithsonian Institution, Washington, D.C.; MZSP, Museu de Zoologia da Universidade de São Paulo.

RESULTS

Genus *Calliostoma* Swainson, 1840
(For further discussion and synonymy, see Marshall (1995a))

Calliostoma adpersum
(Philippi, 1851)

Figs. 1–8, 36–39, 45–47, 54–77

Trochus eximius Reeve, of Philippi, 1844: 17, pl. 4, fig. 7 (*non* Reeve, 1842).

Trochus adpersus Philippi, 1849: pl. 32, fig. 8; 1851: 217.

Ziziphinium adpersum Beck, *in litteris*, Philippi, 1851: 218.

Calliostoma adpersum: Tryon, 1889: pl. 18, figs. 1, 2; 1890: 367; Quinn, 1992: 107, figs. 98–100, 121; Sunderland & Sunderland, 1996: 16; Salvador et al., 1998: 1016; Abbott & Dance, 1986: 38, fig.; Rosenberg, 2009; Souza et al., 2010: 306.

Calliostoma (Kombologion) adpersum: Clench & Turner, 1960: 46, pl. 30; Rios 1970: 25, pl. 4; 1975: 23, pl. 5, fig. 58; 1985: 21, pl. 9, fig. 84; 1994: 33, pl. 9, fig. 95; 2009: 50, fig. 108; Regteren Altena, 1975: 10.

Type Material: Not found.

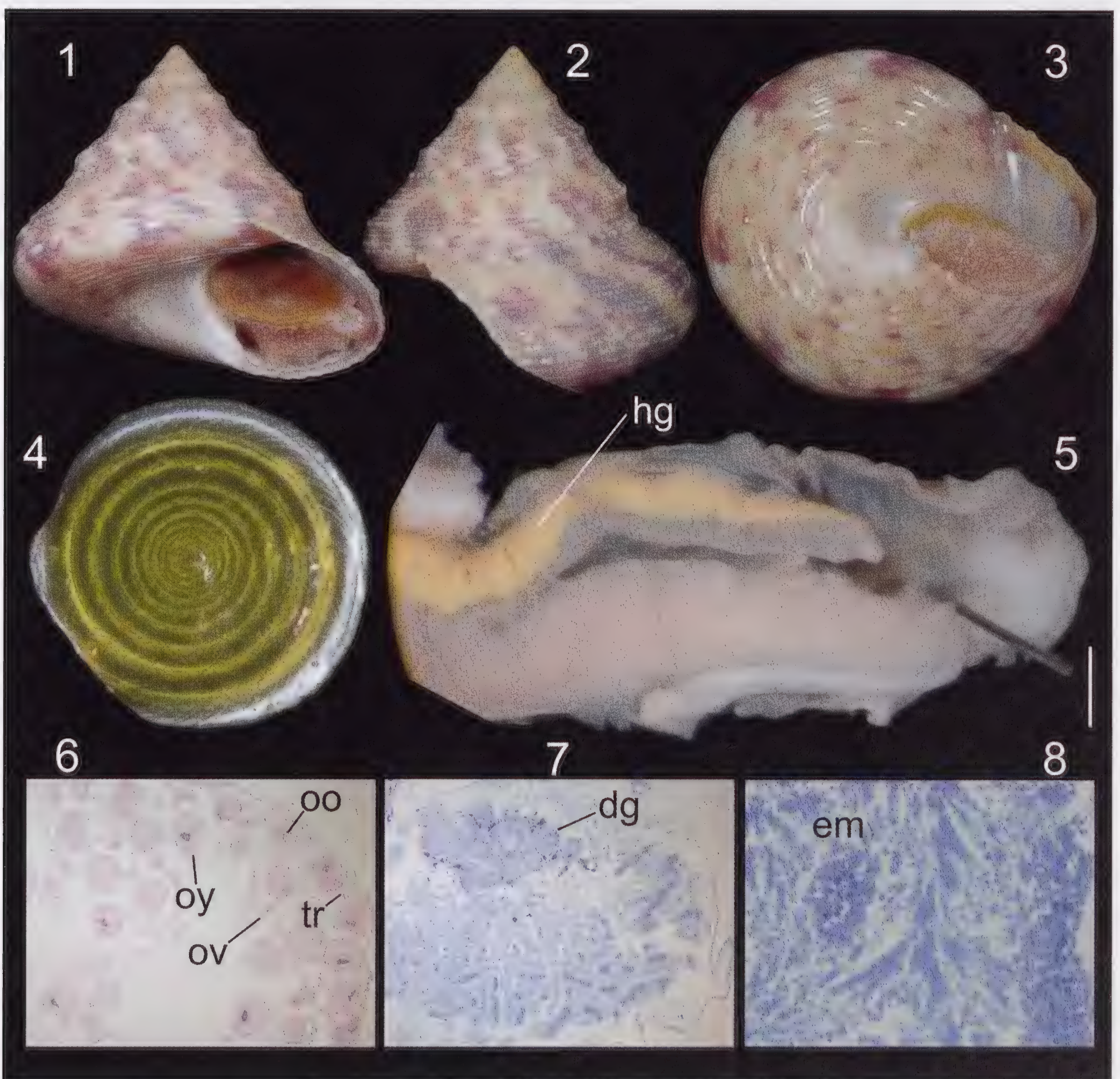
Type Locality: Brazil.

Remarks

According to some authors (Clench & Turner, 1960; Quinn, 1992), the type specimen of *Calliostoma adpersum* was deposited in the MfN, but is likely lost. The type locality was given only as Brazil by Philippi. Later, Clench & Turner (1960) "restricted" [ICZN Code Recommendation 76A: "ascertaining or clarifying"] the

locality to Praia do Leste, Ilha Guaíba, State of Rio de Janeiro. A neotype designation would take precedence over this.

We will not assign a neotype for *C. adpersum* because the search for the holotype is still ongoing. According to Dance (1986: 222) "Philippi's specimens described in his *Enumeratio Molluscorum Siciliae* (1836–44) and some others in the Berlin Museum [MfN]. Some types in the Brit. Mus. [Natural History Museum, London]. Presumably the coll. made after 1851 is in Santiago Mus. Chile". We only checked the holdings of the MfN and the NHM.

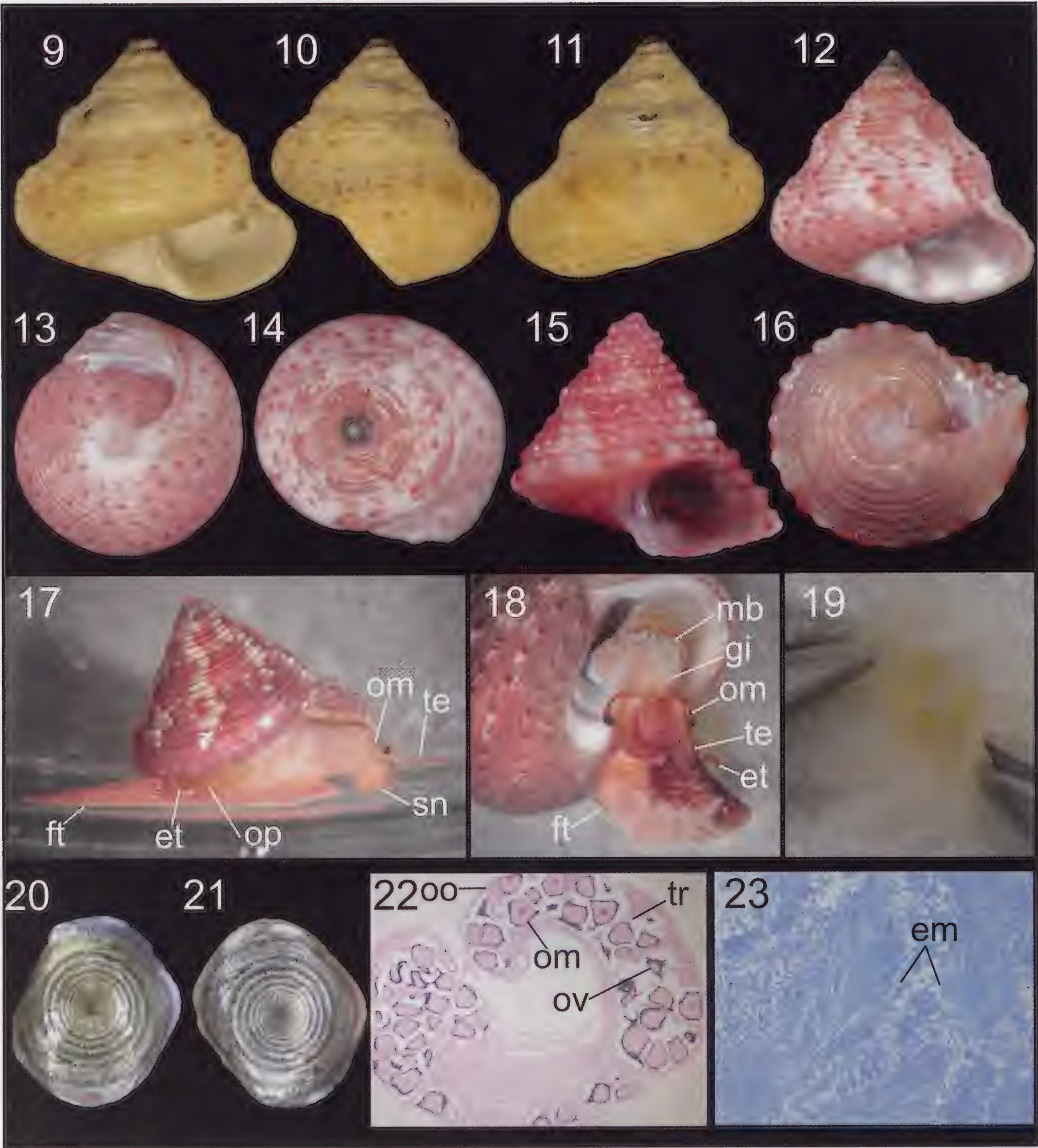


FIGS. 1–8. *Calliostoma adpersum*. FIGS. 1–3: Shells in frontal, lateral and umbilical views, MZSP 94332 (length 16.5 mm); FIG. 4: Operculum internal view, MZSP 24117 (diameter 5.6 mm); FIG. 5: Pallial cavity, ventral-inner view, MZSP 38193 (scale bar = 1 mm); FIG. 6: Ovary, MZSP 38190; FIGS. 7, 8: Testis, MZSP 63562 (HE; 5 µm).

Diagnosis

Shell flat sided with flattened keel at whorl periphery. Foot highly papillate, light brown-

ish colored. Orange hypobranchial gland on female. Ventral esophageal fold twice as larger than dorsal folds. Spiral caecum with 1.5 whorls.

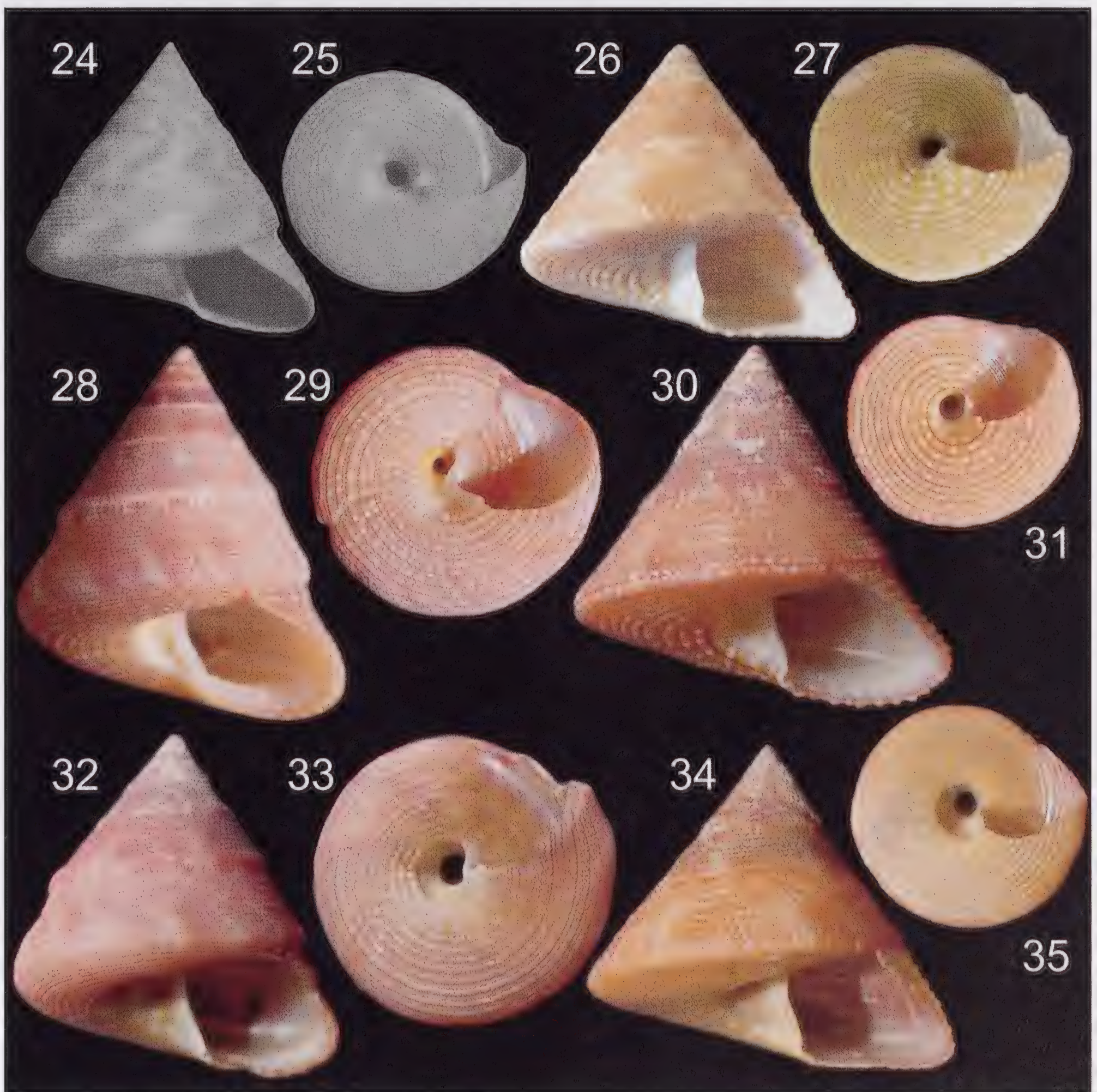


FIGS. 9–23. *Calliostoma depictum*. FIGS. 9–11: Holotype NMNH 152267 (length 10 mm), courtesy NMNH; FIGS: 12–14: Shells in frontal, umbilical and apical views, MZSP 66727 (length 13.3 mm); FIGS. 15, 16: Shells of juveniles in frontal and umbilical views, MZSP 92390 (length 2.5 mm); FIGS. 17, 18: Living specimen, MZSP 92413 (length ~10 mm); FIG. 19: Jaws in situ, ventral view, MZSP 41575 (length ~ 1 mm); FIG. 20: Operculum, internal view; FIG. 21: Same, external view, MZSP 41575; FIG. 22: Ovary, MZSP 47575; FIG. 23: Testis, MZSP 41575 (HE; 5 µm).

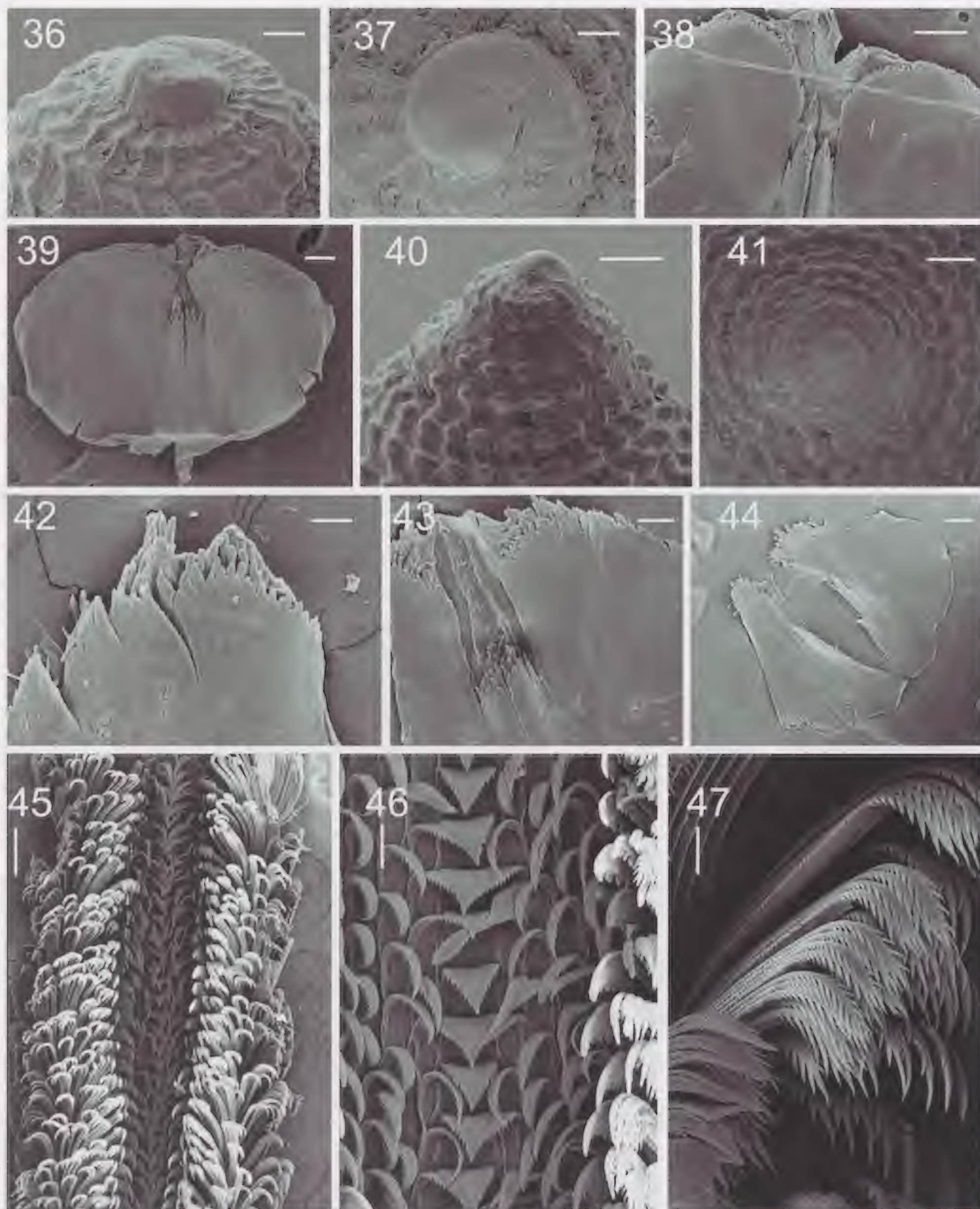
Description

Shell (Figs. 1–3): up to eight whorls and 20 mm in height and diameter; imperforate and grooved (Fig. 3), depressed trochoid, flat-sided with flattened keel on last adult whorl. Color ivory, with large axially aligned irregular brownish red or purple blotches; basal portion with irregularly spaced, fine reddish brown or purple spots on cords. Protoconch (Figs. 36, 37) of one whorl, light-colored, smooth.

Suture indistinct. Umbilical area iridescent, with a lengthened depression to left of columella. Teleoconch sculptured by beaded spiral cords; superior cord more pronounced along whorls. Last adult whorl with 18 beaded cords. Keel with two broad beaded cords, with two smaller cords between them. Basal surface of last adult whorl with 12 smooth and large cords. Thin ribs (Fig. 36) between beaded cords in first teleoconch whorl. Spire angle $\sim 80^\circ$. Aperture subquadrate; proso-



FIGS. 24–35. Shells of *Calliostoma hassler* in frontal and umbilical views. FIGS. 24, 25: Holotype MCZ 104554, extracted from Clench & Turner (1960) (length 30 mm); FIGS. 26, 27: Holotype of *C. vinosum* MORG 29294 (length 13.5 mm), courtesy MORG; FIGS. 28, 29: MZSP 37264 (length 30 mm); FIGS. 30, 31: MZSP 37264 (length 17.5 mm); FIGS. 32, 33: MZSP 34885 (length 27.5 mm); FIGS. 34, 35: MZSP 34855 (length 20 mm).



FIGS. 36–47. Protoconch, jaws and radulae in SEM. FIGS. 36, 37: *Calliostoma adpersum* protoconch in slanted and apical views, MZSP 41738 (scale bars = 60 μ m); FIGS. 38, 39: Same, jaws in ventral view, MZSP 24117 (scale bar = 200 μ m); FIGS. 40, 41: *Calliostoma depictum* upper whorls with oblique and apical views, MZSP 66835 (scale bars = 200 and 300 μ m); FIGS. 42, 43: Same, jaws in detail of anterior area, MZSP 91749 (scale bars = 40 and 60 μ m); FIG. 44: Jaws of *Calliostoma hassler*, ventral view, MZSP 34885 (scale bar = 300 μ m); FIG. 45: Radula of *Calliostoma adpersum*, whole view of middle region, MZSP 38190 (scale bar = 200 μ m); FIG. 46: Same, detail of central area, MZSP 38193 (scale bar = 60 μ m); FIG. 47: Same, detail of marginal teeth, MZSP 24117 (scale bar = 40 μ m).

cline ($\sim 45^\circ$ with columellar axis). Columella arched, truncate at base.

Head-Foot (Figs. 54–58): total length slightly more than half whorl when contracted and twice that when extended. Head bulging approximately in middle region of head-foot. Snout wide, cylindrical; distal end wider than base; distal surface plane, papillated, with papillae longer in edge and smaller close to mouth. Each papillae long, cylindrical, with rounded tip. Outer lip of mouth

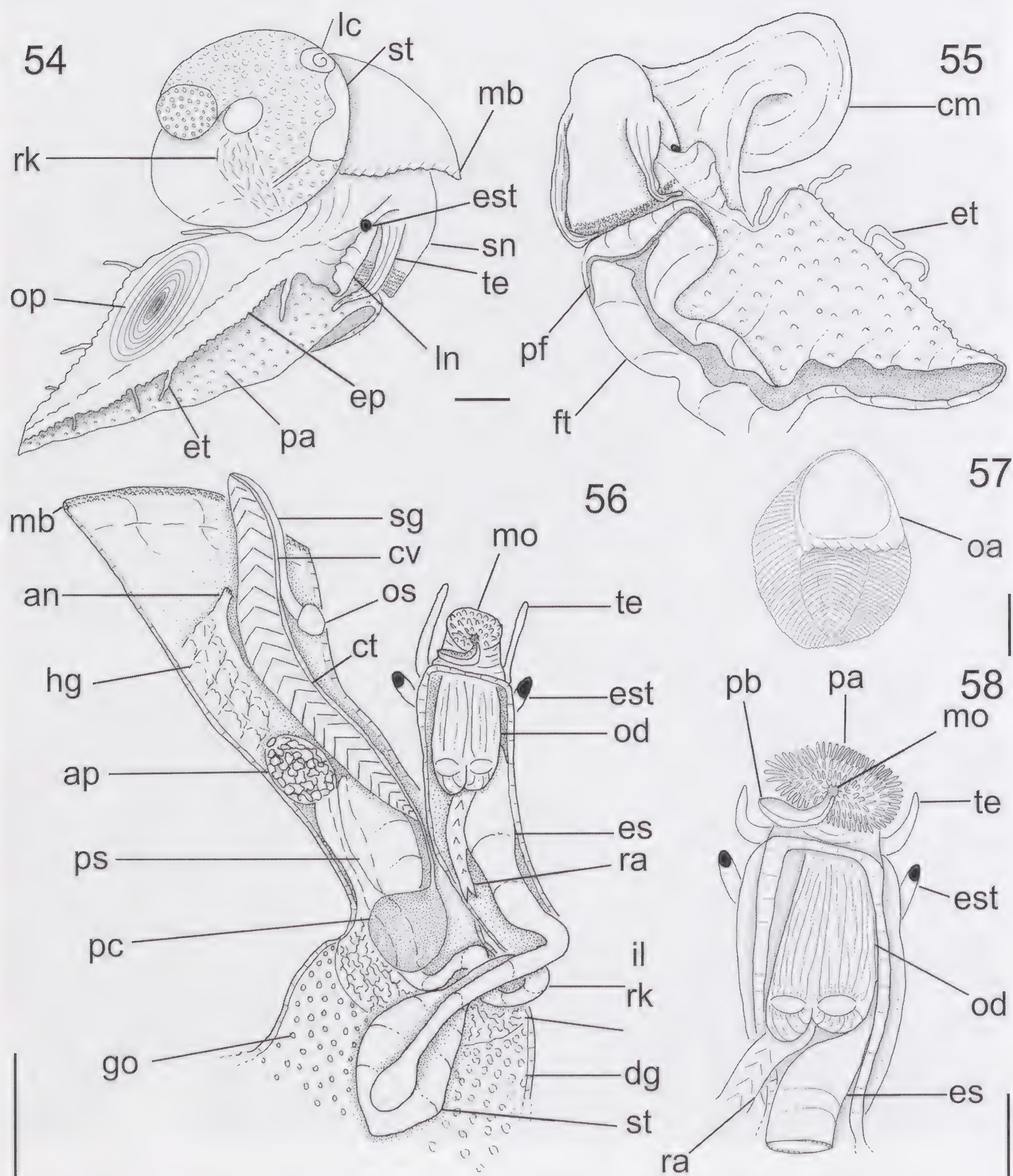
(Fig. 58) with pseudoproboscis. Mouth longitudinal, located in middle portion of ventral surface of the snout. Pair of cephalic lappets absent. Cephalic tentacles (Figs. 54–56, 58: te) $\sim 35\%$ longer than snout, sometimes asymmetrical in relation to one another, covered by small papillae, dorso-ventrally flattened, grooved, narrowing gradually up to a lightly pointed tip. Ommatophore on outer base of cephalic tentacles $1/5$ of tentacles length. Eyes dark, round, occupying anterior edge of ommatophore.



FIGS. 48–53. Radulae in SEM. FIG. 48: *Calliostoma depictum*, whole view in middle region, MZSP 91749 (scale bar = 200 μm); FIG. 49: Same, detail of central area, MZSP 41575 (scale bar = 60 μm); FIG. 50: Same, detail of central area and marginal teeth, MZSP 92320 (scale bar = 60 μm); FIG. 51: *Calliostoma hassler*, detail of central area, MZSP 69424 (scale bar = 200 μm); FIG. 52: Same, detail of rachidian and lateral teeth, MZSP 69424 (scale bar = 60 μm); FIG. 53: Same, detail of marginal teeth, MZSP57485 (scale bar = 90 μm).

Foot thick, occupying half of total head-foot length; dorsal region light brown densely papillated. Foot divided into mesopodium and epipodium. Epipodium (Fig. 54: ep) surrounding latero-dorsal region of mesopodium, equidistant from sole and base of ommatophores.

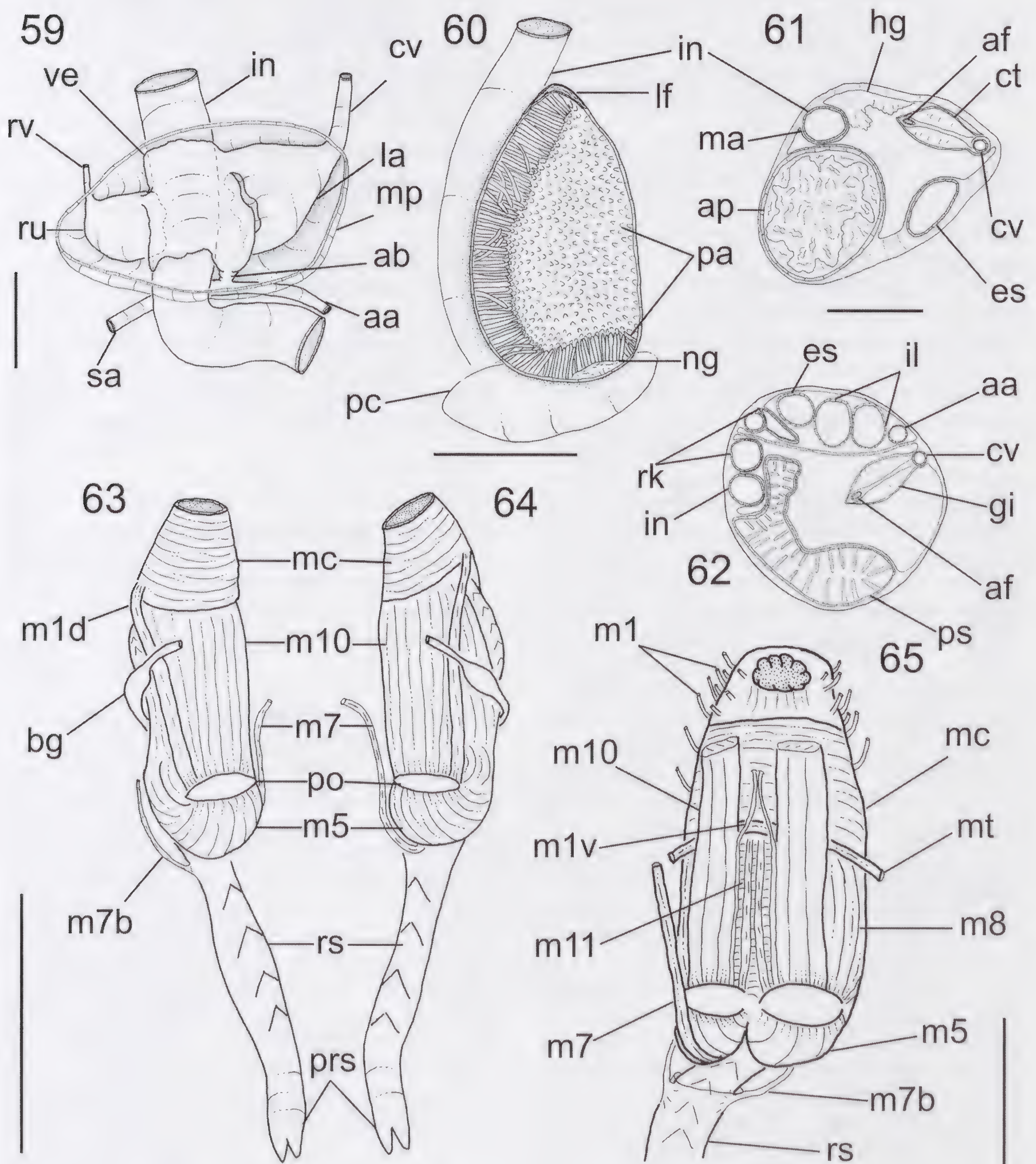
Opercular pad (Fig. 57) rounded, located in median dorsal region, with free edge on posterior area; posterior end with several chevron furrows, apex pointed toward posterior and two pairs of longitudinal furrows on median line. Furrow of pedal glands along entire anterior



FIGS. 54–58. Anatomy *Calliostoma adpersum*. FIG. 54: Head-foot, right side and visceral mass; FIG. 55: Same, anterior-slightly left view; FIG. 56: Pallial cavity roof, head, head-foot haemocoel and visceral mass, ventral view; FIG. 57: Opercular pad, dorsal view; FIG. 58: Head and haemocoel, ventral view, foot extracted. Scale bars = 2 mm.

edge of foot (Fig. 55: pf); single aperture of pedal glands located in median region of this furrow. Anterior third of epipodium formed by neck lobe (Figs. 54, 55: ln): one pair of wide horizontal flexible flaps. Remaining 2/3 of epipodium relatively low, thick; 4 pairs of slender tentacles (Figs. 54, 55: et) inserted on

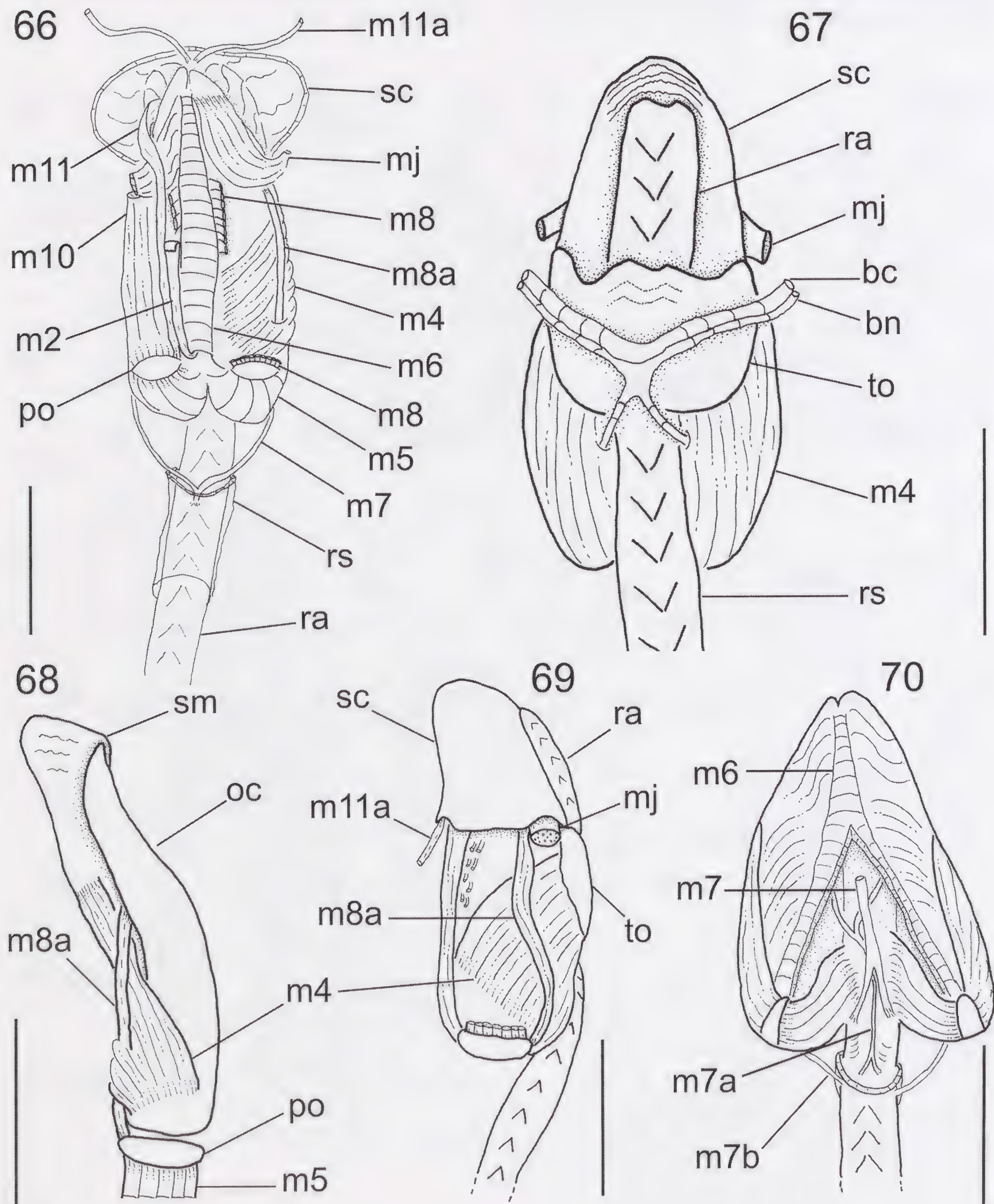
each side at distal edge. Epipodial tentacles located equidistantly, well-spaced from each other, distributed symmetrically on both sides along epipodium; tentacles shorten towards posterior region. Pair of columellar muscles thick, 1/4 whorled and fused with each other along median line.



FIGS. 59–65. Anatomy *Calliostoma adpersum*. FIG. 59: Pericardium, ventral-inner view, ventral walls and kidneys removed; FIG. 60: Papillary sac opened longitudinally, ventral-inner view, right kidney removed; FIG. 61: Transverse section in middle of ampulla region; FIG. 62: Transverse section in middle of right kidney (scale bars = 1 mm); FIGS. 63, 64: Buccal mass in right and left views; FIG. 65: Buccal mass in ventral view (scale bars = 2 mm).

Operculum (Figs. 4, 54): about 6 mm in diameter and 12 whorls, closing entire aperture; thin, with a central nucleus. Corneous, yellowish gold in color. Inner side convex, outer side concave.

Mantle Organs (Figs. 5, 56): pallial cavity of 3/4 whorl. Mantle border (Fig. 56: mb) thick, white; anterior end papillated, occupying 1/3 of mantle border. Gill located on left side of



FIGS. 66–70. Odontophore *Calliostoma adpersum*. FIG. 66: Ventral view, subradular cartilage opened longitudinally and deflected, m8 and m10 of left side removed; FIG. 67: Same, dorsal view; FIG. 68: Buccal mass, right side in lateral view, m8 and m10 removed, subradular membrane and m5 deflected; FIG. 69: Left view, m5, m8 and m10 removed; FIG. 70: Ventral view, m6 opened longitudinally along median line. Scale bars = 2 mm.

pallial cavity, measuring $3/4$ of its length and projecting anteriorly and sustained by rod of gill and suspensory membrane (Fig. 56: sg). Anterior end of gill narrow, with pointed tip. Afferent gill vessel $\sim 3/4$ of gill's length, originating in transverse pallial vessel and running on distal region of central axis of gill. Transverse pallial vessel $\sim 1/5$ of afferent vessel length, originating in left nephrostome and discharging in afferent gill vessel. Ctenidial vein (efferent gill vessel) 35% longer than afferent vessel, running on basal region of central axis of gill; posterior end of vein ($1/10$ its length) free from gill filaments, lying parallel (on the left) to afferent vessel up to the pericardium. Hypobranchial glands (Figs. 5, 56: hg) on both sides of rectum; undeveloped and white in male; thicker and orange in female.

Rectum occupying $1/4$ of pallial cavity width, sigmoid; posterior region under kidneys. Anus siphoned, preceded by pleated and short free end, located on anterior right side of pallial cavity, close to mantle border. Kidneys $\sim 1/3$ of rectum length, located on posterior region of pallial cavity; right kidney modified on females (see below).

Visceral Mass (Figs. 7, 54, 56, 72): pericardium and posterior portion of right kidney exposed in pallial cavity roof. Stomach and spiral caecum (Figs. 54, 72) $1/3$ whorl posterior to pallial cavity. Digestive gland on left side and gonad on right side (Figs. 7, 56: dg, go), both posterior to right kidney.

Circulatory and Excretory Systems (Figs. 56, 59–62): pericardium located between pallial cavity and visceral mass (Fig. 56: pc), close to median line and immediately posterior to kidneys. Left side of pericardium receiving ctenidial vein and right side receiving right pallial vein (Fig. 59: cv, ru). Ventricle $1/3$ of pericardium volume; surrounding rectum and flanked anteriorly by left auricle (ventral, triangular, of $1/3$ of pericardium volume) and posteriorly by right auricle (weak, smaller than left). Bulb of aorta (Fig. 59: ab) emerging from left end of ventricle and forming anterior and posterior aortas (Fig. 59: aa, sa). Anterior aorta running forward to end in series of sinuses in both head and foot. Posterior aorta running up to visceral hump.

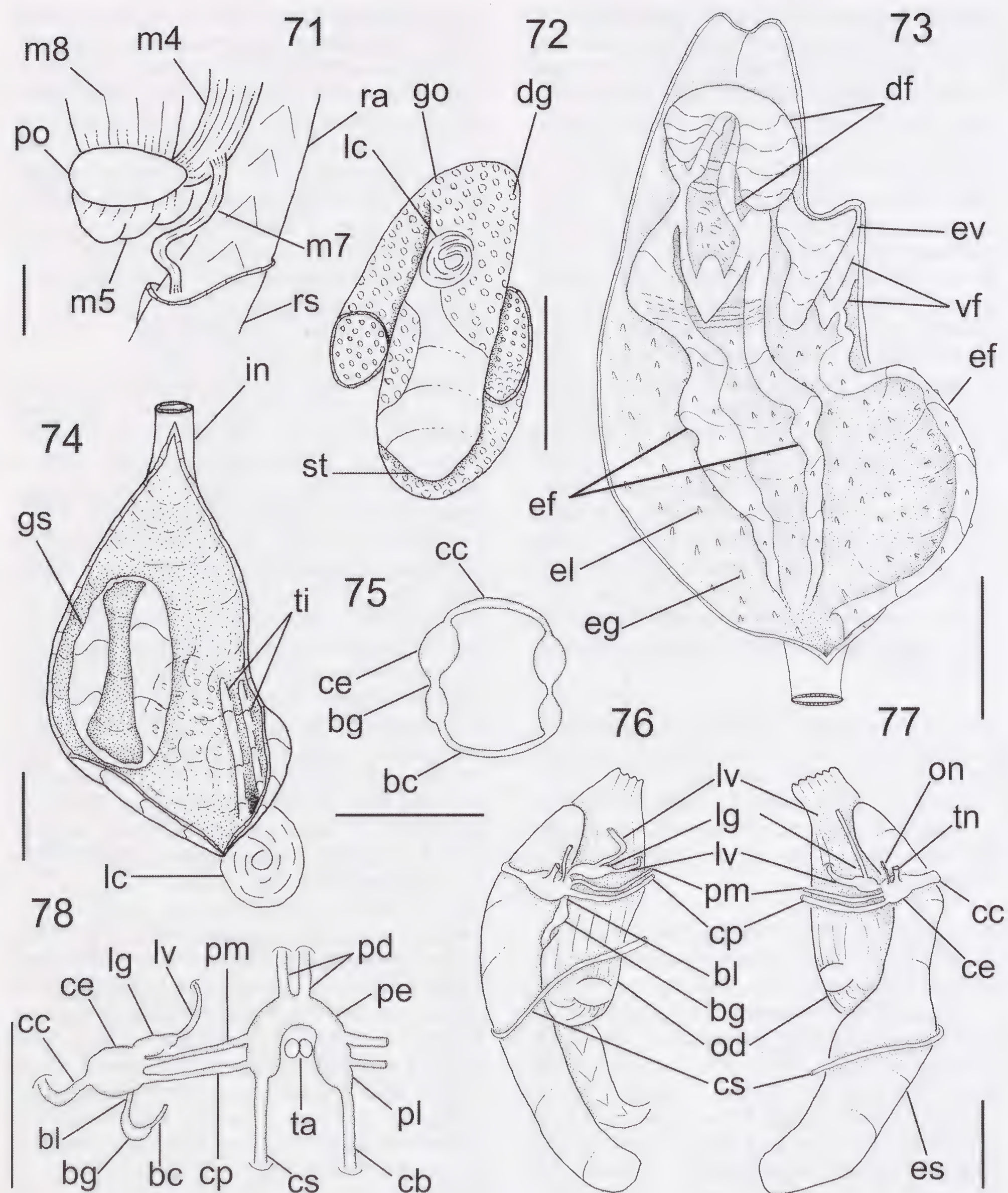
Papillary sac (Figs. 56, 60, 62: ps), or left kidney, $1/3$ of rectum length; base oval, wide, gradually narrowing towards anterior portion, ending at left nephrostome; inner wall covered with numerous thin, long papillae; posterior end

connecting with pericardium by nephridial gland (Fig. 60: ng), as special thickening of tissue.

Right kidney (Fig. 56: rk) divided into two regions. Anterior region a hollow tube $1/3$ of rectum length and $1/2$ the width of the papillary sac in males. In females ampulla, or urogenital papilla, (Figs. 56, 61: ap) large (75% larger than papillary sac), oval, hollow, filled by mucus. Posterior region spreading around visceral mass immediately beneath mantle, encircling inner surface of columellar muscle. Kidney expanding ventrally to cover half of right surface of adjacent visceral hump (Fig. 62).

Digestive System (Figs. 5, 7, 38, 39, 45–47, 56, 58, 63–74, 76, 77): oral tube length $\sim 1/2$ odontophore length; walls with circular muscles (Figs. 63, 64: mc); basal region with oblique fibers (Fig. 66: mj), originating gradually from dorsal surface, close to median line. Fibers running posteriorly towards both sides, inserting in ventral surface of odontophore. Jaw plates (Figs. 38, 39) thick, light brown, rounded, occupying half of buccal cavity dorsal surface. Minute denticles on ventral surface, starting on anterior portion and running obliquely towards ventral portion. Anterior edge denticulate, without true fringe. Pair of dorsal folds starting posteriorly to jaws (Fig. 73: df), with each fold bending and forming two partially overlapping rounded slits. Ventral slit triangular. Series of transverse muscles separating outer surface of esophagus from odontophore. Odontophore about twice longer than snout.

Odontophore muscles (Figs. 63–71): m1: series of small jugal muscles connecting buccal mass with adjacent inner surface of snout and haemocoel. m1d: pair of small dorsal jugal retractors, originating in postero-lateral region of mouth sphincter and inserting in antero-lateral edge of posterior cartilage. m1v: pair of small ventral jugal protractors of odontophore, originating on central surface of oral tube, running posteriorly away from each other and inserting in postero-ventral region of odontophore, in median surface of posterior cartilages. m4: broad pair of dorsal tensor muscles of radula and subradular membrane, originating partly in ventral surface of anterior cartilages, at some distance from median line, and partly in posterior lateral surfaces of posterior cartilages, surrounding lateral and ventral surfaces of anterior cartilages, with insertion along dorsal region of subradular membrane (exposed inside buccal cavity) and a portion in radular ribbon preceding buccal cavity. m5: pair of large accessory dorsal tensor muscles of radula, originating in ventral



FIGS. 71–78. Anatomy *Calliostoma adpersum*. FIG. 71: Left view of posterior region of odontophore, radular sac partially removed (scale bar = 1 mm); FIG. 72: External dorsal view of stomach and adjacent region of visceral whorls; FIG. 73: Buccal cavity, anterior and mid-esophagus opened longitudinally, ventral-inner views, odontophore removed; FIG. 74: Stomach opened longitudinally, ventral-inner view (scale bars = 2 mm); FIG. 75: Overview of nerve ring (scale bar = 1 mm); FIGS. 76, 77: Foregut and central nervous system, right and left sides (scale bar = 2 mm); FIG. 78: Topology of nervous system, frontal view (scale bar = 1 mm).

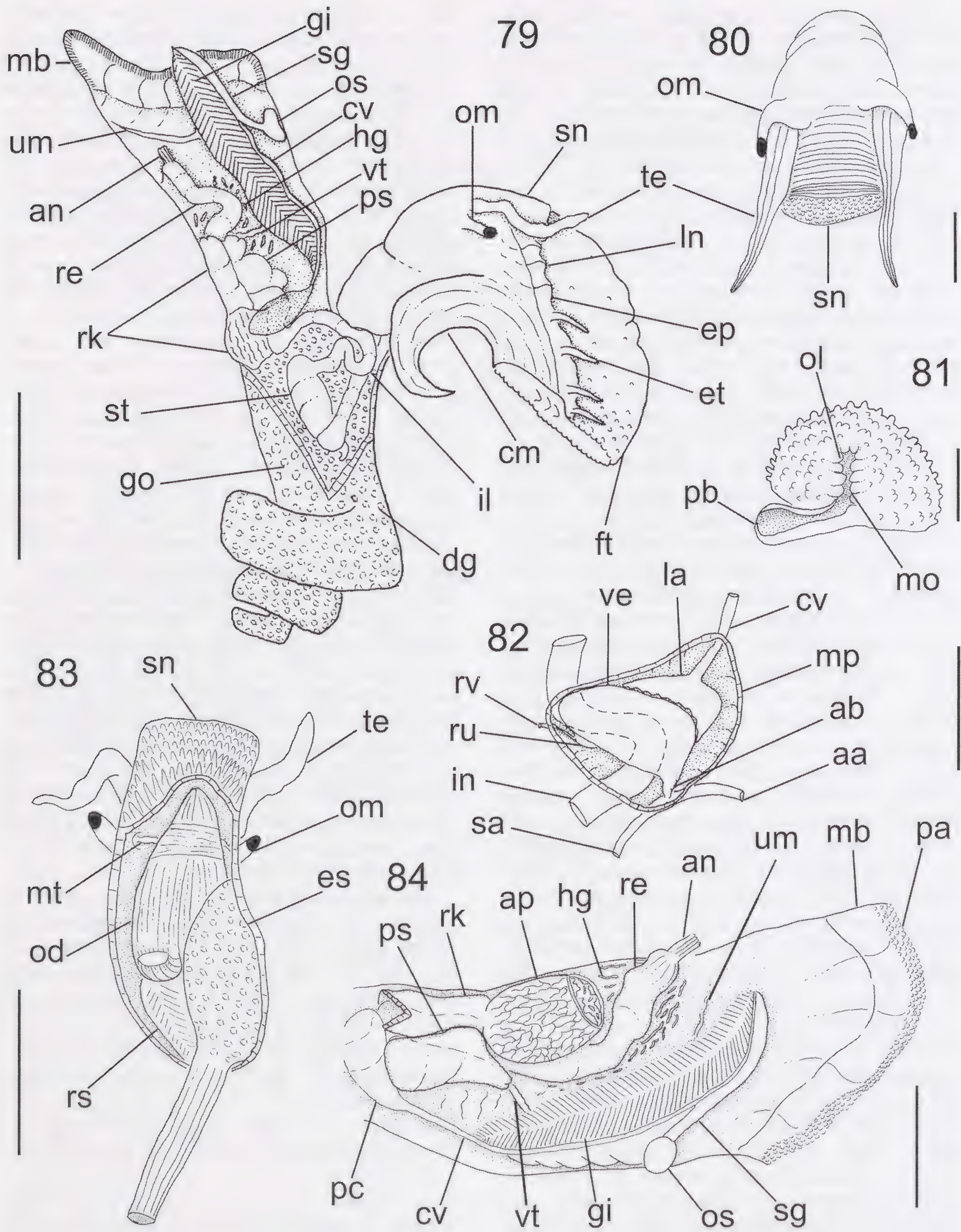
surface of posterior cartilages, running firstly towards dorsal and median and subsequently anteriorly, with insertion in region of radular ribbon. m6: horizontal muscle uniting almost entirely the ventral edges of the two anterior cartilages (excepting short posterior region on external surface). m7: very small, thin pair of muscles, originating on middle region of inner ventral surface of radular sac, running anteriorly and separating from one another at their middle level (insertion not observed). m7a: very small and thin pair of muscles, originating in haemocoelic ventral surface, running dorsally and separating from one another surrounding m7, with insertion in radular sac, posterior to insertion of m5. m7b: pair of muscles originating in lateral inner wall of radular sac, inserting in posterior cartilage. m8: pair of broad approximator muscles of cartilages, originating in anterior cartilages (along its lateral surface and posterior to insertion of mj), running posteriorly, decreasing gradually and inserting in middle region of anterior surface of posterior cartilages; m8a, pair of accessory muscles of m8, originating posteriorly in anterior cartilages on the opposite side of m8 origin, running parallel, covering m4 and inserting in antero-lateral region of posterior cartilages. m10: broad pair of ventral protractor muscles of odontophore, originating from ventral region of mouth and buccal sphincter, running posteriorly, inserting in anterior region of posterior cartilage, ventrally to m8 insertion. m11: pair of thin ventral tensor muscles of radula, originating in middle region of ventral surface of posterior cartilage (separated from one another by distance equivalent to their width), running anteriorly and covering m6 and ventral surface of anterior cartilage, becoming wider in anterior region and inserting in distal edge of subradular membrane. m11a; very long and thin pair of oblique ventral tensor muscles of radula, originating in haemocoelic anterior surface near pleural ganglia, running dorsally between anterior edge of anterior cartilages and inserting in subradular membrane distal edge. mj: originating gradually from dorsal surface close to median line, running divergently towards posterior and sides and inserting in ventral surface of odontophore. mt: single transversal muscle originating from lateral wall of haemocoel, running through ventral surface of odontophore under m10 and m1v and inserting into opposite wall.

Non-muscular structures of odontophore (Figs. 66–68): oc: pair of anterior cartilages,

antero-posteriorly elongated, flat, with anterior and posterior ends rounded and of same length of odontophore. po: small pair of posterior odontophore cartilages, of 1/10 of anterior cartilages length and located in posterior region, slightly lateral to anterior cartilages. br: subradular membrane covering most of the odontophore surface exposed in buccal cavity, where most of intrinsic odontophore muscles inserts. sc: subradular cartilage maintaining radular ribbon, expanding in buccal cavity beyond radula and covering about half the exposed portion of subradular membrane.

Radular sac wide, twice longer than odontophore. Posterior end of radular sac broadly bifid. Radula (Figs. 45–47) symmetrical, rows arched. Rachidian 1/9 of radula width, base rectangular; cutting edge with projection turned posteriorly (almost 90°) and covering posterior end of preceding tooth; tip narrowly tapered, finely serrated; interlock sub-terminal, at base of cutting projection. Four lateral teeth similar to rachidian but narrower and bent medially, forming curve with concave side towards radular sac; teeth sharply pointed, finely serrated, with about 25 secondary cusps separated from each other along both edges. About 30 marginal teeth; innermost pair greatly enlarged, with large base and coarsely serrate hooked cusps; secondary cusps curved, on inner surface twice size of cusps on outer surface. Succeeding inner marginals of similar built, but less massive. Outer marginals with delicate secondary cusps along both sides of tip; become more slender towards the edge of radula.

Inner surface of anterior esophagus with three food channels, two dorsal and one ventral (Fig. 73: df, vf), the latter bearing esophageal valve (Fig. 73: ev). Mid esophagus with three folds (two dorsal and one higher ventral) forming three shallow chambers; epithelium (Fig. 73: el, eg) entirely covered by villous papillae. Posterior esophagus (Fig. 56) narrow (but with enlarged posterior portion), with some thin longitudinal folds on inner surface. Stomach (Figs. 56, 72, 74: st) oval, very thin walled, taller than wide, occupying 1/4 of first whorl, divided into two parts, a more globular posterior and a more cylindrical anterior. Posterior part with two typhlosoles on left side (Fig. 74: ti) running into spiral caecum; gastric shield (Fig. 74: gs) a thin cuticle with 1/6 of stomach area, located on middle left side of posterior part. Spiral caecum (Figs. 72, 74: lc) with 1½ counter clockwise (in dorsal view) whorls. Esophageal insertion in posterior me-



FIGS. 79–84. Anatomy *Calliostoma depictum*. FIG. 79: Pallial cavity roof and visceral mass in ventral view, head-foot deflected to right (scale bar = 2 mm); FIG. 80: Head, anterior view; FIG. 81: Detail of anterior surface of snout, ventral view; FIG. 82: Pericardium, ventral-inner view, ventral walls and kidneys removed (scale bars = 1 mm); FIG. 83: Head and anterior digestive system, ventral view; FIG. 84: Pallial cavity roof, ventral-inner view (scale bars = 2 mm).

dian stomach region. Intestine origin in anterior end. Intestine (Fig. 56: in) very wide, running anteriorly forward next to haemocoel, bending abruptly and forming wide loop (Fig. 56: il); anterior region of visceral mass with small loop surrounding kidney and pericardium, exiting in right-posterior corner of pallial cavity. Rectum and anus described above (pallial organs).

Genital System (Figs. 6–8, 56): female with modified right kidney (described above). Brief histology: single epithelium with granular glandular cells; mesothelium composed of connective tissue and muscle fibers. Invagination of epithelium forming radial arrangement of trabeculae. Oogenesis: oogonia of 40 µm (Fig. 6: oo) attached to trabeculae, nucleus central; oocyte (Fig. 6: ov), under development, of 80 µm, pyriform, attached to trabeculae by peduncle, nucleus central; mature oocyte (Fig. 6: om) of 210 µm, surrounded by chorion layer, nucleus and nucleolus displaced from center. Spermatogenesis: germinative tissue attached to trabeculae; mature spermatocyte (Fig. 8: em) with spermatozoa distributed throughout testis as feathery bundles; acrosome, nucleus and flagellum not examined.

Central Nervous System (Figs. 67, 75–77): nerve ring surrounding anterior half of buccal mass. Cerebral ganglia rounded, located on lateral region of buccal mass (Figs. 76, 77: ce), occupying ~ 1/3 of its area; commissure thick, long, dorso-ventrally flattened; cerebropleural and cerebropedal (Figs. 76–78: cp, pm) connectives long, thin, originating in anterior region of cerebral ganglia and running ventrally and back to pedal and pleural ganglia. Labial ganglia (Figs. 76–78: lg) 1/3 of cerebral ganglia, located in ventro-lateral region of buccal mass, anteriorly to cerebral ganglia; connected to cerebral ganglia by short cerebrolabial connective (Fig. 75: cl). Buccal ganglia (Figs. 76, 78: bg) of same size as labial ganglia, posterior to cerebral ganglia; connected to cerebral ganglia by a buccolabial connective (Figs. 77, 78: bl). Buccal commissure thick (Fig. 67: bc, to), lying dorsally in relation to radular tissue. Pleural and pedal ganglia (Fig. 78: pe, pl) close to one another, inside pedal musculature immediately below ventral surface of haemocoel; both of about half size of cerebral ganglion. Pedal commissure thick, very short. Pedal nerve (Fig. 78: pd) running forward from each pedal ganglion, surrounding medial pedal blood

sinus. Supra-esophageal connective (Fig. 78: cs) emerging from right pleural ganglia. Sub-esophageal connective (Fig. 78: cb) emerging from left pleural ganglia. Statocysts (Fig. 78: ta) rounded, bright, located very close to posterior side of pedal ganglia.

Distribution

From Colombia to Brazil, Santa Catarina: 12.27°N to 27°S, 74.5°W to 29.3°W (Rosenberg, 2009).

Habitat

From 20 to 50 m depth, hard bottoms in western part of its range, sandy mud bottom in eastern and southern part.

Material Examined

Brazil: Espírito Santo: Vitória, MZSP 66724, 03 shells (iii/2000). Rio de Janeiro; Arraial do Cabo, MZSP 72425, 1 shell (P. Gonçalves col. i/2005); Búzios, Ossos Beach, 22°46'49.00"S, 41°53'09.82"W, MZSP 62544, 1 shell (Simone col. 18/i/1982); Angra dos Reis, Ilha Grande, MZSP 24110, 10 shells (De Fiore col.). São Paulo: Ubatuba, Boqueirão, MZSP 24111, 05 specimens (14/vii/1962); Anchieta Island, MZSP 26953, 01 specimen; Pesca and Selinha Islands, 23°23'S, 45°52'W, MZSP 38190, 01 specimen (BIOTA – FAPESP 11/vi/2001); Caraguatatuba, Ponta Grossa, 23°37'S, 45°24'W, MZSP 38196, 1 specimen (BIOTA – FAPESP 24/iv/2001); MZSP 38193, 2 specimens (BIOTA – FAPESP 16/x/2001); São Sebastião, MZSP 8958, 22 shells (Ihering col. 1896); Araçá, MZSP 24117, 11 specimens (15/ix/1962), Baraqueçaba Beach, MZSP 24118, 35 shells (E. Boffi col. xi/1969); Ilha-bela, Perequê, MZSP 63562, 2 specimens (Simone 05/vi/1993), MZSP 58934, 1 specimen (Amaral, C. col. 23/ix/1992); Santos, MZSP 71948, 1 shell (v/2004); Peruíbe, Central Beach, MZSP 62554, 3 shells (M. Borges col. 26/i/1982).

Calliostoma depictum

Dall, 1927

Figs. 9–23, 40–43, 48–50, 79–90

Calliostoma depictum Dall, 1927: 6. Rios, 1975: 23, pl. 6; Quinn, 1992: 110, figs. 101, 102, 122; Rosenberg, 2009.

Calliostoma (Kombologion) adpersum Philippi: Clench & Turner, 1960: 46–48, pl. 31; Rios, 1970: 25; Abbott & Dance, 2000: 38 (*non* Philippi, 1851).

Calliostoma (Neocalliostoma) depictum: Rios, 1985: 22, pl. 10, fig. 89.

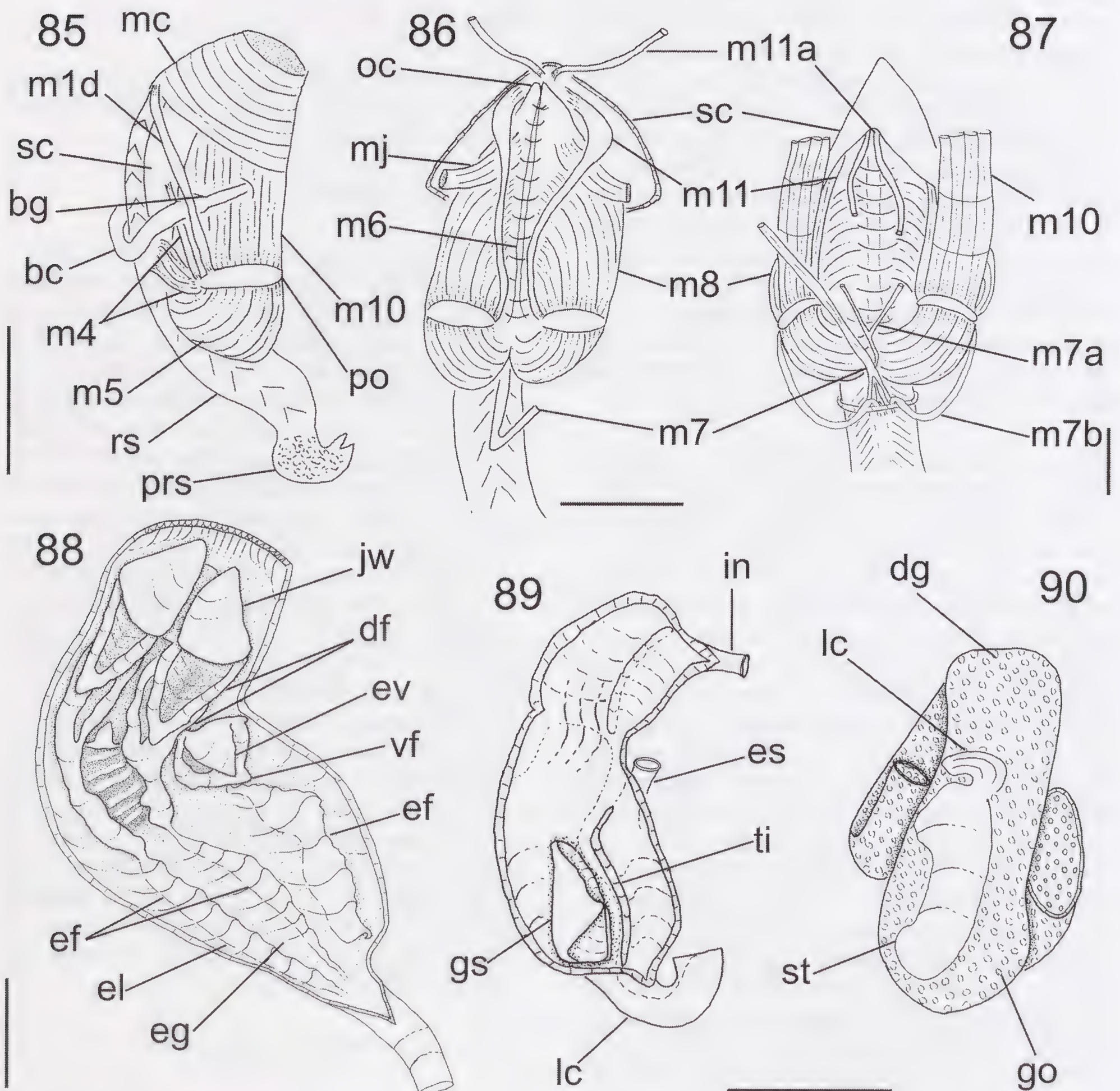
Calliostoma (Emerlinia) depictum: Rios, 1994: 33, pl. 33, fig. 91; 2009: 49, fig. 107.

Type Material: Holotype NMNH 152667 (examined).

Type Locality: Brazil, Bahia State.

Diagnosis

Turbiniform shell, suture distinct. Sculptured by beaded fine threads. Spots of reddish purple color on cords of teleoconch and base. Marked groove near columella. Foot reddish brown. m8a odontophore muscle absent. Ventral esophageal fold slightly larger than dorsal folds. Spiral caecum with 1/2 whorl.



FIGS. 85–90. Anatomy *Calliostoma depictum*. FIG. 85: Buccal mass, right view, anterior and mid-esophagus extracted; FIGS. 86, 87: Odontophore, ventral view. FIG. 86: Subradular cartilage opened longitudinally and deflected, m10 removed; FIG. 87: m10 deflected to show inner structures; FIG. 88: Buccal cavity, anterior and mid-esophagus opened longitudinally, ventral-inner views, odontophore removed (scale bars = 1 mm); FIG. 89: Stomach opened longitudinally, ventral-inner view; FIG. 90: External dorsal view of stomach (scale bars = 2 mm).

Comparative Description

Shell (Figs. 9–18): size ~ 8 mm in height and diameter; 5–6 convex whorls; spire angle ~ 80°. Turbiniform, imperforate, with marked groove near columella (Fig. 13). Color pale with large reddish purple or reddish pink axial patches; some reddish brown or purple spots on cords of teleoconch and base. Apex very dark brown. Protoconch (Figs. 40, 41) of about 1 whorl; structure not observed. Umbilical area white. Suture deep. Teleoconch with spiral beaded cords; last adult whorl and base with 11 cords, smoother at base; delicate and thin (Fig. 40) ribs in first whorl. Aperture subquadrate, prosocline (~ 40° with columellar axis). Juvenile specimens (Figs. 15, 16) with flattened whorls; colored spots only in innermost line of base. More details in Dall (1927) and Quinn (1992).

Head-Foot (Figs. 17, 18, 79–81): rounded head; snout wide, cylindrical, red-brown, papillated. Outer lip of mouth with pseudoproboscis. Pair of cephalic lappets absent. Cephalic tentacles asymmetrical with each other; dorsal-ventrally flattened, grooved, twice snout length; red brown with darker median strip, white near base. Ommatophore reddish, of about 1/4 the length of cephalic tentacles. Eyes dark, round, occupying anterior edge of ommatophore. Foot strongly reddish brown colored; papillae white. Fewer papillae than preceding species. Opercular pad located in the middle of dorsal region. Barely noticeable chevrons on dorsal region. Furrow of pedal glands along entire anterior edge of foot. Symmetrical neck lobes, with smooth edges. Epipodium with four pairs of tentacles, inserted sub-terminally at its distal edge; tentacles similar in size.

Operculum (Figs. 20, 21): as in preceding species, but brownish silvery, very thin, with 3 mm in diameter and up to 14 whorls.

Mantle Organs (Figs. 79, 84): gill with half palial cavity length and 1/4 of its width, extended anteriorly by wide suspensory membrane. Afferent gill vessel of almost same length as gill. Ctenidial vein 20% longer than gill, free from gill filaments, located left of afferent vessel up to pericardium. Hypobranchial glands (Fig. 84: hg) white, slightly thicker in females. Rectum thick, sigmoid, occupying 2/3 of cavity length. Kidneys 1/3 rectum length. Right kidney modified on females.

Visceral Mass (Figs. 79, 90): similar to preceding species.

Circulatory and Excretory Systems (Figs. 79, 82, 84): ventricle 1/2 of pericardium volume; left auricle 1/4 of pericardium; right auricle ~ 1/5 of left auricle. Vessels as preceding species. Papillary sac 1/3 of rectum length and twice its width. Anterior right kidney with same size of papillary sac on males. Ampulla, on females, with twice the width of papillary sac. Posterior right kidney as preceding species.

Digestive System (Figs. 19, 42, 43, 48–50, 79, 83, 84–90): oral tube (Figs. 85, 86) walls with circular muscles and jaw muscles on basal region. Jaws triangular in shape (Figs. 19, 42, 43); denticles long, without true fringe, on distal edge. Odontophore partially covered by mid-esophagus in ventral view (Fig. 83). Odontophore muscles (Figs. 85–87): m1; m1d; m1v; m4; m5; m6; m7; m7a; m7b; m8; m10; m11; m11a; mj; mt; ef. Radula (Figs. 48–50): rachidian 1/9 of total width; stem wide; cutting edge triangular with projection turned posteriorly (almost 90°); tip tapered, with finely small secondary cusps on both sides. Five lateral teeth with wide bases and very thin stems (1/10 of base width). About 25 marginal teeth. Innermost marginal (Fig. 50) greatly enlarged with heavy three secondary cusps. Other marginals with delicate secondary cusps along both sides of tip; becoming more slender towards the outside.

Buccal cavity and anterior and posterior esophagus similar to preceding species: anterior esophagus with three food channels, two dorsal and one ventral, continuing to mid esophagus (Fig. 88: ef); ventral esophageal fold slightly larger than dorsal folds. Stomach ~ 1/2 visceral mass width (Fig. 90); central region depressed; with two typhlosoles, one on left side of posterior area, other on left side of anterior area. Gastric shield (Fig. 89: gs) thicker than in *C. adspersum*. Spiral caecum 1/2 whorl counter clockwise (Fig. 89: lc). Intestine similar to preceding species.

Genital System (Figs. 22, 23): sexual dimorphism by ampulla on female (see above). Gonad with single epithelium above layer of connective tissue and muscle fibers, forming radial arrangement of trabeculae. Oogenesis: oogonia and oocyte attached to trabeculae, nucleus central; mature oocyte displaced from trabeculae and surrounded by chorion layer,

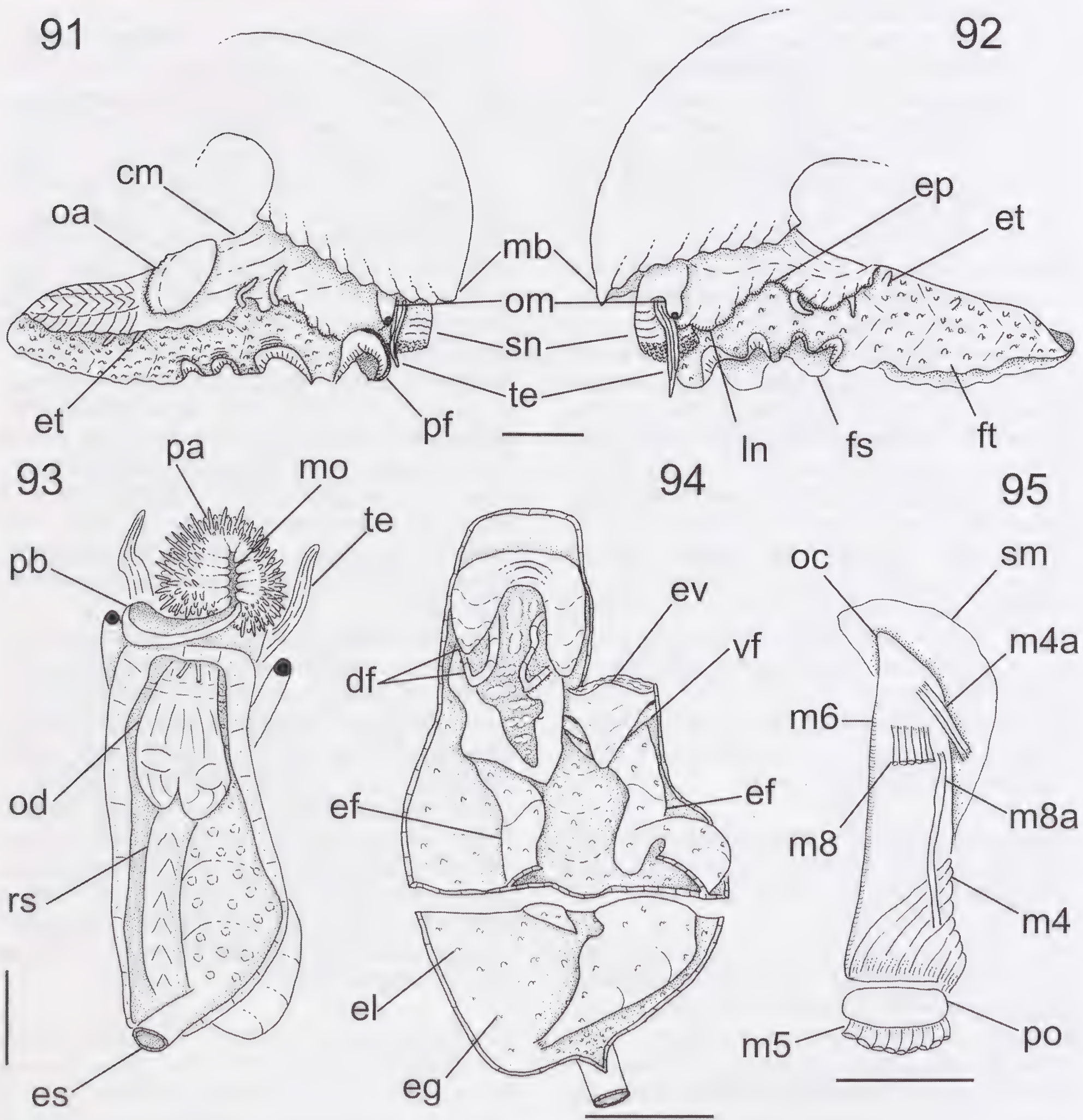
nucleus and nucleolus displaced from center. Spermatogenesis: germinative tissue attached to trabeculae and spermazoa feather-like.

Central Nervous System: nerve ring located on anterior half of buccal mass. Cerebral ganglia rounded, dorso-ventrally flattened. Cerebropleural connective twice as thick as cerebropedal connective. Labial ganglia 1/5 of cerebral ganglia; cerebrolabial connective as

thick as cerebropleural. Buccal ganglia slightly larger than labial ganglia; buccal commissure thick. Pleural and pedal ganglia close to one another; connective imperceptible. Statocysts rounded, bright.

Distribution

Brazil, Maranhão to São Paulo: 13°S to 23°S, 43°W to 29.3°W (Rosenberg, 2009).



FIGS. 91–95. Anatomy *Calliostoma hassler*. FIG. 91: Head-foot and adjacent region of mantle in right view; FIG. 92: Same in left view (scale bars = 4 mm); FIG. 93: Head and anterior digestive system, ventral view, foot removed; FIG. 94: Buccal cavity, anterior and mid-esophagus opened longitudinally, ventral-inner views, odontophore removed; FIG. 95: Odontophore, left side in lateral view, m8 and m10 removed, subradular membrane and m5 deflected (scale bars = 2 mm).

Habitat

From 1 to 7 m, in sandy areas around rocks and coral. Often found below sea urchins.

Material Examined

Holotype: NMNH 152667.

Brazil: Rio Grande do Norte: Rio do Fogo, MZSP 84265, 1 shell (M. Coltro, col. i/2007). Bahia; MZSP 56354, 1 shell; Salvador, MZSP 71951, 1 shell (B. Linhares col. xi/1998), MZSP 66727, 2 shells (B. Linhares col. vii/1998), Farol da Barra Beach, MZSP 81797, 3 shells (Simone col. 22–28/ii/1997). Rio de Janeiro: Cabo Frio, Papagaio Island, 22°46'S, 41°53'W, MZSP 92320, 11 specimens (Dornellas col. 14/ix/2009); Arraial do Cabo, Saco do Cherne, 22°53'37.6"S, 41°59'13.8"W, MZSP 92318, 1 specimen (Dornellas col. 15/ix/2009), Forno Beach, MZSP 92413, 1 specimen (Dornellas col. 18/ix/2009); Angra dos Reis, Jorge Grego Island, 23°13'S, 44°08'W, MZSP 91749, 27 specimens (Dornellas & Amaral col. 08/viii/2009). São Paulo: São Sebastião, Ilhabela, Cabras Island, 23°49'S, 45°23'W, MZSP 41575, 9 specimens (Simone et al. col. 06/v/2004); Alcatrazes Island, Faroleiros Harbor, MZSP 28322, 2 specimens (Simone col. 29/xi–01/xii/1996); Santos, Embaré Beach, MZSP 84604, 1 shell (Simone col. 1978); Itanhaém, Queimada Pequena Island, MZSP 84887, 4 specimens (Dornellas et al. col. 01/iii/2007).

Calliostoma hassler

Clench & Aguayo, 1939

Figs. 24–35, 44, 51–53, 91–98

Calliostoma (Astele) hassler Clench & Aguayo, 1939: 191, pl. 28, fig. 3.

Calliostoma hassler – Clench & Turner, 1960: 66–67, pl. 48; Rios, 1970: 25, pl. 5; 1975: 24, pl. 6, fig. 64; 1994: 34, pl. 10, fig. 101; 2009: 51, fig. 114; Abbott & Dance, 1986: 38, fig.; Rosenberg, 2009.

Calliostoma (Elmerlinia) bullisi Clench & Turner – Rios, 1970: 24, pl. 5 (*non* Clench & Turner, 1960).

Calliostoma barbouri Clench & Turner – Rios, 1975: 23, pl. 5, fig. 60 (*non* Clench & Aguayo, 1946).

Calliostoma (Neocalliostoma) hassler – Rios, 1985: 22, pl. 10, fig. 91.

Calliostoma vinosum Quinn, 1992: 96, figs. 53–56, 114; Rios, 2009: 53, fig. 118, syn. nov.

Type Material: *Calliostoma hassler* Holotype in MCZ 104554. Paratypes in MCZ 104555 and Museo Poey; *Calliostoma vinosum* Holotype in MORG 29.294. Paratypes in USNM 860256; FSBC I 44067; MORG 15.043.

Type Locality: Brazil, Rio de Janeiro off Cabo Frio.

Diagnosis

Shell with rounded peripheral keel. Foot strongly reddish brown colored, densely papillated on dorsal region. Rachidian slender, with two pronounced flaps on its base. Two esophageal folds. Intestine forming two wide loops; spiral caecum absent.

Comparative description

Shell (Figs. 24–35): up to 25 mm in height and diameter and eight slightly convex whorls; spire angle ~ 65°. Trochoid, flat sided, finely sculptured, with rounded peripheral keel. Umbilicus deep. Color light reddish brown with yellowish white axial blotches. Base with white colored spots on cords. Protoconch of one whorl, light-colored. Umbilical area yellowish to white. Sculptured by beaded spiral cords; last adult whorl and base with 15 cords; inner cord on base stronger than the others. Suture indistinct. Aperture subquadrate, prosocline (~ 45° with columellar axis of shell). Columella arched, not truncate at base, forming slight callus. Juvenile specimens (Figs. 26, 27, 30, 31, 34, 35) with flatter whorls and without rounded peripheral keel.

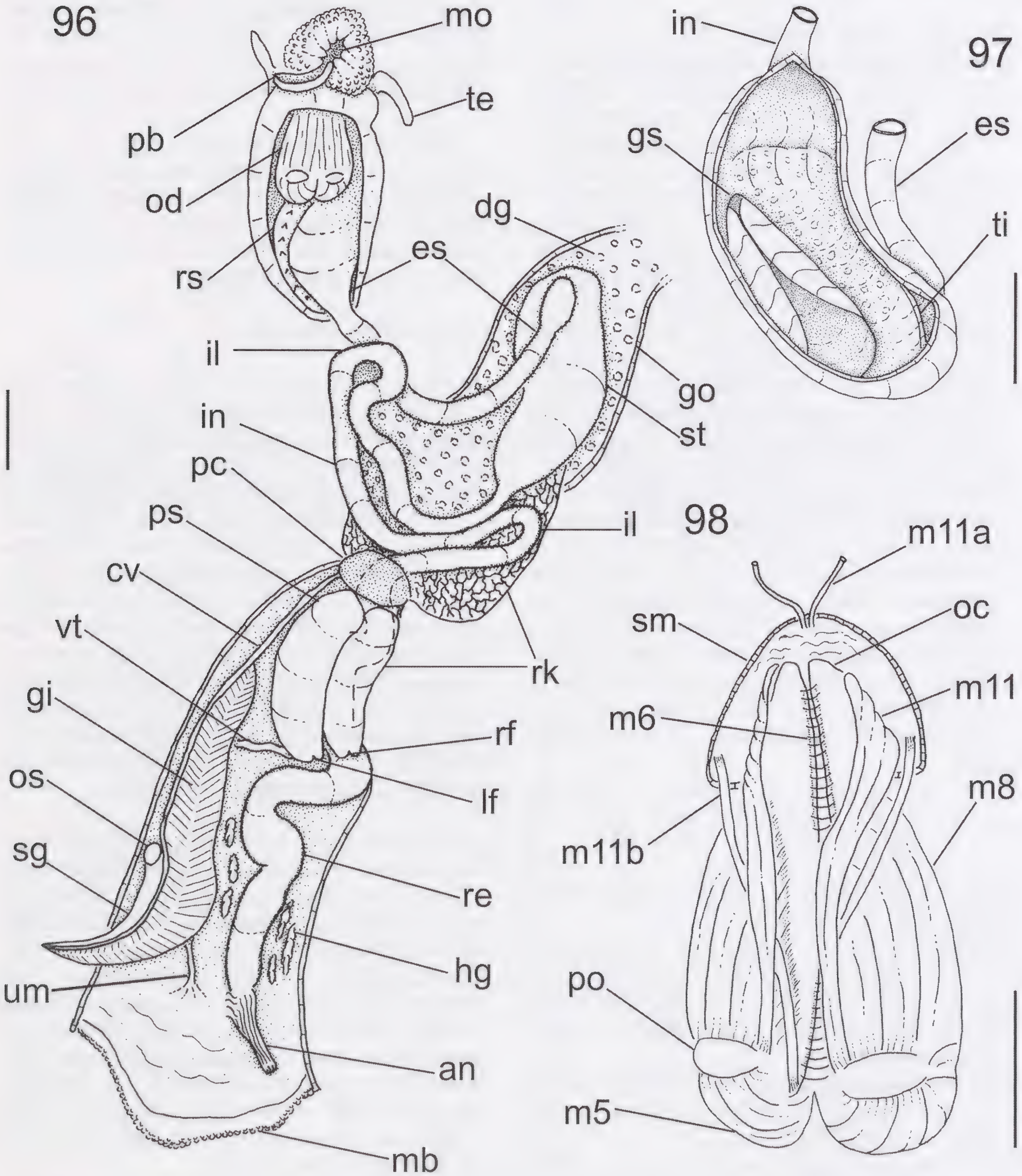
Head-Foot (Figs. 91–93): snout 1/3 of head-foot width; distal end densely papillated; outer lip with pseudoproboscis. Cephalic lappets absent. Cephalic tentacles dorsal-ventrally flattened, grooved, covered by small papillae. Ommatophore 1/3 of tentacles length. Foot brown with numerous white papillae on dorsal region; posterior end (Fig. 91) light brown with chevrons and two tall longitudinal furrows. Neck lobes asymmetrical: left lobe finely digitated; right lobe with smooth edge. Epipodium with 4 pairs of tentacles (Figs. 91, 92: et), nearly equidistant to each other.

Operculum: similar to *C. adpersum*. 12 whorls and 8 mm in diameter.

Mantle Organs (Fig. 96): gill with more than half of pallial cavity width; afferent vessel ~ 2/3 gill

length. A quarter of the ctenidial vein free from gill filaments. Hypobranchial glands thin, white. Rectum thick, sigmoid, occupying almost entire cavity length.

Visceral Mass (Fig. 96): 1/4 of first whorl and remaining whorls. Otherwise similar to preceding species.



FIGS. 96–98. Anatomy *Calliostoma hassler*. FIG. 96: Pallial cavity roof, visceral mass, head-foot deflected, ventral-inner views; FIG. 97: Stomach opened longitudinally, ventral-inner view; FIG. 98: Odontophore, ventral view, subradular cartilage opened longitudinally and deflected, m10 and radular sac removed. Scale bars = 2 mm.

Circulatory and Excretory Systems (Fig. 96): pericardium not examined. Papillary sac and anterior right kidney 1/3 of rectum length and same width. Posterior right kidney as in preceding species.

Digestive System (Figs. 44, 51–53, 93–98): oral tube walls with circular muscles; jaw muscles on basal region. Jaws (Fig. 44) triangular in shape, dark brown; very long denticles on anterior end, projected in tufts. Odontophore muscles (Figs. 95, 98) similar to preceding species (except for m11b): m1, m1d, m1v, m4, m5, m6, m7, m7a, m7b, m8 and m8a as in *C. adpersum*; m10, m11, m11a, mj, mt. Muscle m11b: thin ventral pair of radular muscles originating in dorsal surface of posterior cartilage, running parallel to m11 and inserting itself in ventral surface of anterior region of subradular membrane. Radula (Figs. 51–53): rachidian 1/10 of total radula width, with very large nearly triangular base and narrow stem. Six lateral teeth, with base larger and stem shorter from those of preceding species; two outer teeth modified in lateromarginal plates. About 30 marginal teeth; innermost teeth similar to *C. adpersum*, remaining teeth similar to one another; secondary cusps becoming slender and larger; central cusp rounded.

Buccal cavity and esophagus similar to both preceding species, except for mid esophagus with two esophageal folds (Fig. 94: ef): a higher ventral fold and a right dorsal fold. Stomach (Fig. 97) without spiral caecum; one typhlosole on left side of posterior area; gastric shield similar to *C. depictum*. Intestine longer than in preceding species; following same route, but forming two wide loops (Fig. 96: il). Anus wider.

Genital System: not examined.

Central Nervous System: cerebral ganglia rounded; commissure long, thick, dorso-ventrally flattened. Cerebropleural connective ~ 1/3 cerebropedal width. Labial ganglia ~ 1/5 cerebral ganglia; cerebrolabial connective short. Buccal ganglia about same size as labial ganglia; buccal commissure thick. Pleural and pedal ganglia not observed.

Distribution

Venezuela to Rio de Janeiro, Brazil: 4°N to 23°S, 63°W to 32°W (Rosenberg, 2009).

Habitat

From 20 to 100 m; living on bottoms where bryozoans and coralline algae are abundant (Clench & Turner, 1960).

Material Examined

Brazil: Bahia; Belmonte, MZSP 37264, 3 specimens (A. Bodart col. vii/2003); Alcobaça, MZSP 34885, 11 specimens (Coltro leg.). Espírito Santo; Guarapari, 5 specimens (vi/2006), MZSP 69424, 09 specimens (A. Bodart col. v/2004), MZSP 57485, 9 specimens (Coltro leg. iv/1992).

DISCUSSION

The anatomy of *Calliostoma adpersum*, *C. depictum* and *C. hassler*, as well as *C. zizyphinum* (Linnaeus, 1758), *C. jujubinum* (Gmelin, 1791) and *C. tupinamba* Dornellas, 2012 (Dornellas, 2012; Fretter & Graham, 1962; Sá & Coelho, 1986), when compared with other morphological studies on trochoids or vetigastropods, revealed unique anatomical particularities. As such, the particularities of *Calliostoma* according to some authors (Randles, 1905; Fretter & Graham, 1962), and corroborated here, are: presence of ampulla in female (Figs. 84, 56: ap); reduction or lack of cephalic lappets (Figs. 55, 80); presence of pseudoproboscis (Figs. 81, 93: pb). Most vetigastropods have an intestinal loop inside the head-foot haemocoel, but in this study, we observed it outside of the haemocoel (Figs. 56, 79, 96: il). The absence of visceral structures in haemocoel is considered a synapomorphy of Caenogastropoda (Simone, 2011) and is, then, a convergent character that Caenogastropoda share with *Calliostoma*. A typical radular pattern characteristic of Calliostomatinae consists of thin, highly flexible teeth with fine, serrate cusps (Hickman & McLean, 1990), a modified innermost marginal tooth with a heavy base and coarsely serrate hooked cusp (Fig. 50), and the remaining lateral marginal teeth gradually decrease in size.

Nevertheless, the conchological characters lack any attribute that can be considered unique. Despite this, the identification of *Calliostoma* is relatively easy. The trochoid shape, flattened or slightly convex whorls, beaded spi-

TABLE 1. Morphological differences of the shells, jaws and radulae between the three species of *Calliostoma*.

Shells, jaws and radulae attributes	<i>C. adpersum</i>	<i>C. depictum</i>	<i>C. hassler</i>
Whorls	somewhat flattened	rounded convex	slightly convex
Peripheral keel	flattened	without a keel	rounded
Umbilicus	imperforate	imperforate	perforate
Umbilical chink	absent	present	absent
Ontogenetic differences	absent	present	present
Jaws	rounded without a true fringe	pointed without a true fringe	pointed with a true fringe
Lateromarginal plate	absent	absent	present
Lateral teeth	5	4	4

ral cords sculpture, presence of axial blotches, and a subquadrate aperture are also present in other trochoid taxa (Hickman & McLean, 1990; Rios, 1994, 2009). The shell attributes allow the identification of the *Calliostoma* species (Table 1), but they cannot be neatly grouped as genera or subgenera as there are a wide variety of intermediate characters (Marshall, 1995a).

It is possible to detect different conchological patterns during development in *C. depictum* and *C. hassler*. The juveniles of both species have flat whorls (Figs. 15, 16, 26, 27, 30, 31, 34, 35), and the adults of *C. hassler* have a round keel, with a more round profile on the last (Figs. 24, 25, 28, 29, 32, 33). Shells of *C. depictum* have round convex whorls with colored spots on the base and the teleoconch. The juveniles of *C. depictum* have similar spots (Figs. 9–14), found only in the innermost line of the base. These ontogenetic stages are observed in populations of both species and because of this, we conclude that *C. vinosum* (Figs. 26, 27) is in fact a young *C. hassler*. The uniformity of anatomical patterns (discussed below) also confirms that they are the same species, as specimens identifiable as both were studied. *Calliostoma vinosum* is based only on conchological and external morphological features (Quinn, 1992), and was only compared with *C. barbouri* Clench & Aguayo, 1946, with no mention to *C. hassler*.

According to some authors, the neck lobe (Fig. 54: In) of Trochidae is asymmetrical, one neck lobe has a smooth edge and the other with a digitated edge, or one is larger than the other, as is observed in species of *Tegula* Lesson, 1835; *Gibbula* Risso, 1826; and *Anti-*

margarita Powell, 1951 (Randles, 1905; Righi, 1965; Aldea et al., 2009). Symmetrical neck lobes and four pairs of epipodial tentacles are observed in *Calliostoma* species (Randles, 1905; Fretter & Graham, 1977), but an asymmetrical neck lobe was observed in *C. hassler* (Table 2) and many others calliostomatids from western Atlantic, New Zealand and New Caledonia (Quinn, 1992; Marshall, 1995a, b). Four pairs of epipodial tentacles are not unique to *Calliostoma*, and they occur similarly in *Tegula* and *Astraea* Bolten, 1798 (Turbinidae) (Righi, 1965; Monteiro & Coelho, 2002; personal observation).

The pallial organs in *Calliostoma* differ from other vetigastropods by the presence of the ampulla in females (Figs. 84, 56: ap). The hypobranchial glands of females in *C. adpersum* (Fig. 5: hg) are more development and orange. The function of these glands is still not understood. It seems to be associated with the production of an egg ribbon and defensive against possible predators (Fretter & Graham, 1962; Bryan et al., 1997; Harasewych, 2002). In a behavioral study on defense in *Calliostoma canaliculatum* (Lightfoot, 1786), the hypobranchial gland released a viscous yellow substance (Bryan et al., 1997). It should be noted that this colored gland was observed only in females of *C. adpersum*.

The ampulla, also known as urogenital papilla (Hickman, 1992), was described for *C. zizyphinum* by Perrier (1889) and is expanded to the right nephrostome and projected forward and located in a more anterior level than the left nephrostome. Some authors suggest that this structure is unique for the genus (Randles,

TABLE 2. Morphological differences of the soft parts between the three species of *Calliostoma*.

Soft parts attributes	<i>C. adpersum</i>	<i>C. depictum</i>	<i>C. hassler</i>
Neck lobe	symmetrical	asymmetrical	asymmetrical
Orange hypobranchial gland on female	present	absent	absent
m8a	present	absent	present
m11b	absent	absent	present
Esophageal folds	3	3	2
Ventral esophageal fold <i>versus</i> dorsal fold	ventral fold twice as larger	same size	ventral fold twice as larger
Spiral caecum, whorls	1.5	0.5	0
Large intestinal loop	1	1	2

1905; Fretter & Graham, 1962; Sá & Coelho, 1986). The ampulla was observed in *C. jujubinum*, *C. tupinamba* and here in *C. depictum* and *C. adpersum*, but not observed in *C. hassler*. We attribute this to preservational quality and not as an inherent character to *C. hassler*. A modified right kidney on females is present in other vetigastropods, *Gibbula* and *Monodonta* Lamarck, 1799, showed a tumid, fringed edge at the right nephrostome, called rosette (Randles, 1905; Fretter & Graham, 1962; Andrews, 1985). Although Righi (1965) mentioned an ampulla for *Tegula viridula* (Gmelin, 1791), its nephrostome most resembles the rosette, and it is on the same level of the left nephrostome in *T. viridula* and in *Astraea* (Monteiro & Coelho, 2002). On the other hand, some species of *Pleurotomaria* J. Sowerby, 1821, (*Pleurotomarioidea*) have a right nephrostome similar to that of *Calliostoma* ampulla. This structure is named the oviduct by Woodward (1889).

In a review of the western Atlantic *Calliostoma*, Clench & Turner (1960) assigned *C. adpersum* to the subgenus *Kombologion* Clench & Turner, 1960, and diagnosed the subgenus based on jaw and radula attributes (Table 1 shows differences among the three species). Those authors did not assign *C. hassler* to any subgenus, as neither the morphology of the jaws nor the radulae were known. *Calliostoma depictum* was placed in the subgenus *Elmerlinia* Clench & Turner, 1960, by Rios (1994), but its jaw and radula are not like those described for that subgenus (Clench & Turner, 1960: 4–6). Compared to other species, the jaw and radula of *C. hassler* (Fig. 53) appear more similar to those of *C. jujubinum* (allocated in *Elmerlinia*), differing by marginal teeth that do not change along the row. According to the subgenera de-

scriptions by Clench & Turner (1960), there is no one to properly attribute to *C. depictum*.

The odontophore muscles do not differ greatly among the species studied. The m8a (Figs. 68, 69, 95) is only present in *C. hassler* and *C. adpersum*. m11b appears to be exclusively found in *C. hassler* (Fig. 98) and *C. tupinamba*. It should be noted that there is little knowledge on the odontophore of trochoids, since only a few species were studied at this detail (e.g., Fretter & Graham, 1962; Righi, 1965; Simone & Cunha, 2006; Dornellas, 2012), and there are no studies of *Calliostoma* odontophores. At this time, the m1d, m7b and m11a pairs are exclusive to *Monodonta lineata* (da Costa, 1778), *Tegula viridula* and *Calliostoma* (Fretter & Graham, 1962; Righi, 1965), and the m1v is unique to *Gaza* Watson, 1879, and *Calliostoma* (Simone & Cunha, 2006). More research is needed into odontophore anatomy in *Calliostoma*, as well as in other trochoids.

As usual for vetigastropods, the odontophore cartilages of *Calliostoma* has two pairs of cartilage which consist of anterior and posterior pairs. The posterior cartilage is smaller than anterior cartilage and normally is not fused or overlapped, connected by approximator muscles of cartilage (m8). This conformation of cartilage indicates a plesiomorphic condition for gastropods (Katsuno & Sasaki, 2008).

Radula analysis is very useful in the diagnosis of subgenera in *Calliostoma* and at the species level (Clench & Turner, 1960; Castellanos & Fernandez, 1976; Marshall, 1995a, b; Quinn, 1992). The differences between *C. adpersum* and *C. depictum* radulae are described by Quinn (1992: 110) and are supported here. *Calliostoma depictum* has a more broadly cusped rachidian and has fewer pair of lateral

teeth (4 vs. 5, respectively) than *C. adpersum*. Additionally the rachidian have a narrower base and stem of lateral teeth in *C. adpersum* than in *C. depictum* (Figs. 46, 49). The number of marginal teeth reported by Quinn (1992), about 40 versus about 25, is not absolute, once a small difference between these numbers, about 30 versus about 25, can overlap. The radula of *C. hassler* resembles that of *C. jujubinum* (Clench & Turner 1960: pl. 5, fig. 2), but the marginal teeth (Fig. 53) are less different from their neighboring teeth along the row.

The enlarged mid-esophagus of trochids, as well as in other groups of gastropods, is usually composed of esophageal folds which compartmentalize this region, thereby increasing the area of food processing (Fretter & Graham, 1962; Salvini-Plawen & Haszprunar, 1987; Salvini-Plawen, 1988; Sasaki, 1998). The presence of papillate glands (Figs. 73, 88, 94: el, eg) covering the inner wall in this region is one of the main morphological synapomorphies of the clade Vetigastropoda (Haszprunar, 1988; Salvini-Plawen, 1988; Sasaki, 1998). For the mid-esophagus (Table 2), the number of esophageal folds is different in each species. Both *C. depictum* and *C. adpersum* have two dorsal and one ventral fold, being the ventral fold twice larger in *C. adpersum* (Figs. 73, 88: ef), also observed in *C. tupinamba* and *C. zizyphinum*. On the other hand, *C. hassler* has the mid-esophagus with only one dorsal and one ventral fold, which is as large as that in *C. adpersum*. The mid-esophagus of other trochids, as *Tegula viridula* and *Gaza compta* (Simone & Cunha, 2006), and the turbinids, *Astraea latispina* (Philippi, 1844) and *A. olfersii* (Philippi, 1850) is formed by four esophageal folds, two dorsal and two ventral (Righi, 1965; Monteiro & Coelho, 2002; Simone & Cunha, 2006). Currently, this aspect of three esophageal folds seems to be unique for *Calliostoma* and an important character for defining species. Fretter & Graham (1962) demonstrated the importance of a highly detailed study of the stomach anatomy. The proximal portion of the stomach possesses the gastric shield, a chitinous cuticle structure, and it is also characterized by the presence of two folds (typhlosoles). The distal portion is a narrow chamber laying the intestine (Strong, 2003).

The caecum is widely variable among basal gastropods. It varies from a small, crescent shape to a large, spiral sorting-area structure. The large spiral caecum is considered derived within Vetigastropoda. This structure opens

ventrally on the posterior end of the stomach, more or less as continuous extensions of the typhlosoles (Strong, 2003).

Nevertheless, a tendency for disappearance of the spiral caecum (Figs. 74, 89: lc, 97) in *C. adpersum*, *C. depictum* and *C. hassler* was observed, with 1.5, 0.5, and 0 whorls, respectively. *C. zizyphinum* and *C. jujubinum* are more similar to *C. adpersum*, with 1.5 whorls (Randles, 1905; Fretter & Graham, 1962; Sá & Coelho, 1986). Although the number of whorls may vary among species (e.g., there are 4.5 to 6 whorls in *Astraea latispina*, and 3 to 4.5 whorls in *A. olfersii*; Monteiro & Coelho, 2002), the loss of this structure at this level has never been reported. According to Fretter & Graham (1962), the absence or vestigial spiral caecum is found only in advanced gastropod groups. In fact, and pertinent to the midgut observations, it should be noted that the preservation of the visceral mass of trochids depends on techniques rarely used in ordinary collections (Simone & Cunha, 2006; personal observation). It consists basically of making a small incision in the apical portion of the shell in order to allow the alcohol to be absorbed throughout the body. In the absence of these procedures, the study of these structures is difficult. Therefore, we believe there may be some vestigial spiral caecum in the stomach of *C. hassler* where the spiral caecum is not projected outside, somewhat similar to *Monodonta lineata*, (Fretter & Graham, 1962).

The intestine of the studied *Calliostoma* species differs from other trochids (Monteiro & Coelho, 2002; Sasaki, 1998; Simone, 1998) by lacking an intestinal loop (Figs. 56, 79, 96: il) within the head-foot haemocoel. In these species, and in more derived caenogastropods (Simone, 2004, 2005, 2011), there is no intestinal loop or visceral organs in the haemocoel. In *C. hassler* (Fig. 96: il), the intestine has two long intestinal loops, and is longer in proportion when compared to other species.

The only dimorphism in the genital system is in the ampulla of females (as discussed above). Histologically, the gonads of *C. adpersum* and *C. depictum* (Figs. 6–8, 22, 23) are similar to the other gonocoristic prosobranchs, which have only one epithelium and a trabecular system (Collado et al., 2008; Ortiz-Ordoñez et al., 2009).

A ventral position of the pair of pleural ganglia near the pedal ganglion (Fig. 78: pl, pe) is referred to as a hypoathroid condition in the nervous system. This condition is typical in veti-

gastropods (Fretter & Graham, 1962; Ponder & Lindberg, 1996, 1997). The central nervous system of *Calliostoma* is not differentiated among the species and demonstrates the similar pattern of other trochoids (Sasaki, 1998).

Based on anatomical evidence, *C. depictum* is not a synonym of *C. adpersum*, as was advocated by Quinn (1992). Many attributes separating *C. depictum* from other species, mainly conchological ones. Divergent features are the very convex whorls, distinct suture, and a marked groove near the columella. Soft parts differ in the following ways: an odontophore partially covered by the mid-esophagus (Fig. 83), the absence of the pair of odontophore muscles m8a, a ventral esophageal fold of the same size as the dorsal folds (Fig. 88: ef), and a reduced spiral caecum (Fig. 89: lc) with 0.5 whorl. Additionally, *C. depictum* is often found protected below sea urchins (Quinn, 1992; personal observation); there is no record of such behavior for *C. adpersum*.

As stated above, *C. vinosum* is a synonym of *C. hassler*, because specimens identifiable as both species were shown as a single ontogenetic series. Similar ontogenetic patterns are observed in *C. depictum*. Furthermore, specimens identifiable as *C. hassler* and *C. vinosum* lack the left dorsal esophageal fold, a gastric spiral caecum and an intestine forming two wide loops.

This study demonstrates that soft part features are essential for distinguishing *Calliostoma* species and for answer taxonomic questions that may not be resolved using shell and radula characters. The technique used herein can be applied towards understanding the taxonomy of more species in calliostomatids.

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RESEARCH NOTES

A UNIQUE CASE OF BILATERAL HECTOCOTYLIZATION IN THE NORTH PACIFIC GIANT OCTOPUS (*ENTEROCTOPUS DOFLEINI*)

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INTRODUCTION

Life-history information regarding most cephalopod species is somewhat scarce, and information relating anatomical anomalies of cephalopods is even more limited. For octopus species, the few anomalies that have been reported include: arm bifurcations (Smith, 1907; Okada, 1965; Gonzalez & Guerra, 2006), fourth-arm hectocotylus (Robson, 1929), hexapody (Toll & Binger, 1991), septapody (Gleadall, 1989), decapody (Toll & Binger, 1991), and double hectocotylization (Robson, 1929; Palacio, 1973).

Most male octopus have a modified third right arm or hectocotylus that is specialized for passing sperm packets to females. The tip of this specialized arm, known as a ligula, is spoon-shaped bearing a seminal groove ending in a rounded calamus. There are only three species of octopus that typically have the hectocotylized arm on the third left arm instead of the third right: *Scaevurgus unircirrhos*, *Euaxoctopus panamensis* and *Pteroctopus tetracirrhos* (Palacio, 1973).

In over one hundred years of research, double hectocotylization has been reported in only six individual cases in six different species of octopus including: *Eledone cirrhosa* (by Appellöf, 1892), *Octopus briareus* (by Robson, 1929), *Octopus vulgaris* (by Palacio, 1973), *Octopus selene* (by Palacio, 1973), *Octopus chierchiae* (by Roy Caldwell, personal communication) and *Octopus minor* (by Higashide et al., 2007). In each case, the third right arm was hectocotylized, along with an additional arm from the right side of the body (unilateral hectocotylization) or a left arm (bilateral hectocotylization).

This paper presents not only the first documentation of double hectocotylization for the North Pacific giant octopus (*Enteroctopus dofleini* Wülker, 1910), but also the first case of any cephalopod having double hectocotylization not including either the third right or left arm.

METHODS

Over a period of two years, approximately 5,000 male *E. dofleini* were observed in field studies performed at the edge of the Bering Sea shelf, 20 km north and east of Unalaska Island, Alaska (54°04'N, 166°25'W, Fig. 1). In September 2011, a male *E. dofleini* with bilateral hectocotylization was captured in a standard 2.5 m by 2.5 m groundfish pot, frozen and transported to a laboratory in Unalaska for future analysis and description. In October 2011, the octopus was defrosted and examined. The total weight was measured to the nearest 0.1 kilogram, gonad and stomach were weighed to the nearest gram and spermatophores were counted. Each of the arms was measured to the nearest centimeter and the number of suckers was counted. For the two hectocotylized arms, ligula length was measured in centimeters and evaluated as a percentage of the total length of the hectocotylized arm. The external and internal anatomical measurements of this individual were compared to other male *E. dofleini* of approximately the same total weight (n = 21 and n = 10 respectively).

RESULTS

This specimen (UAM Inv 8145), henceforth referenced as R4L4, was morphologically identical to other males of this size class except for the double hectocotylization of the fourth right and left arms (Fig. 2). This octopus weighed a total of 14.1 kg with a stomach weight of 100 g. The gonad weight for R4L4 was 896 g, and no spermatophores were visible upon inspection. The reproductive tract showed the octopus was immature, but developing normally. Both the gonad weight and the maturity status of the individual were considered normal when compared to similarly sized *E. dofleini*. The mean standard arm length, not including hectocotylized arms

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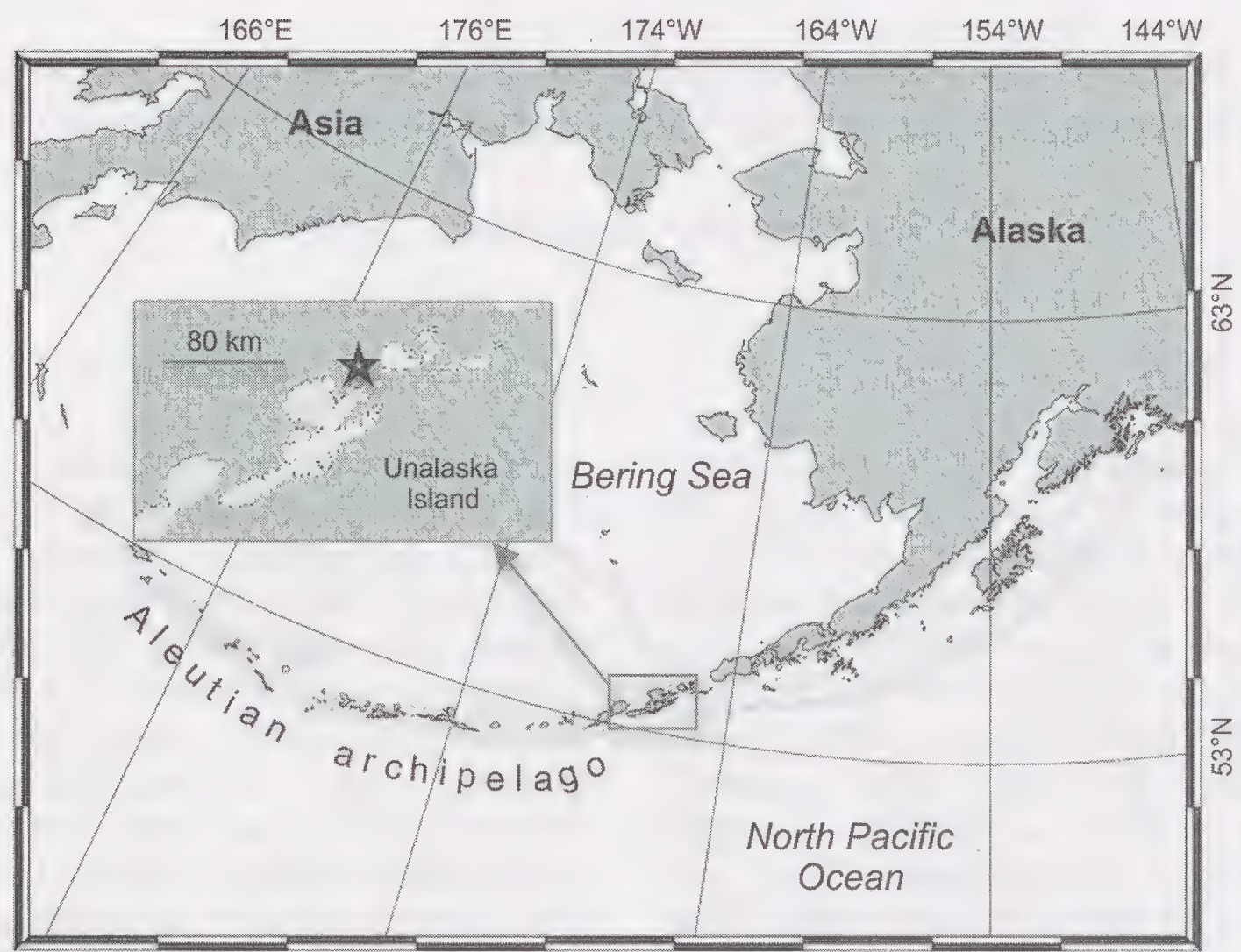


FIG. 1. Capture location (star) of the double hectocotylized *E. dofleini*.

and one arm missing a tip, was 136 ± 0.9 SE cm and the number of suckers per arm was 230 ± 9 SE (Table 1). The length of the fourth right hectocotylized arm, henceforth referenced as R4, was 121 cm and the length of the fourth left hectocotylized arm, henceforth referenced as L4, was 124 cm. The ligula of R4 (29 cm) was 24% of the hectocotylized arm while the ligula of L4 (25 cm) was 20% of the hectocotylized arm.

DISCUSSION

Reports of double hectocotylization and abnormally hectocotylized arms in cephalopods are extremely rare. For all octopus species, only six cases of double hectocotylization and one case of fourth-arm hectocotylization have

been reported (Massy, 1930). Anecdotal reports corroborate this reported rarity as thousands of individuals have been examined by scientists for similar arm anomalies, and negative reports were received for *Octopus maya* and *Octopus vulgaris* in Yucatan, Mexico (Miguel Cabrera, personal communication), *Octopus mimus* in Lima, Peru (Franz Cardoso, personal communication), and *Enteroctopus dofleini* in Canada and Alaska (David Scheel & Jim Cosgrove, personal communication). As such, this individual is unique as both the first documented case of double hectocotylization for this species and for any species of cephalopod that did not include a typical hectocotylized third arm.

Unlike previous cases of reported double hectocotylization, both hectocotylized arms of R4L4 were approximately equal in total length, ligula

TABLE 1. Comparison of arm length, suckers and ligula length for each of the arms of R4L4 (R1 = first right, R2 = second right, etc.).

	R1	R2*	R3	R4 (hecto)	L1	L2	L3	L4 (hecto)
Arm length (cm)	133	99	136	121	137	135	138	124
Suckers	232	94	201	94	220	248	251	104
Ligula length (cm)				29				25

*missing last few cm of arm



FIG. 2. Bilateral hectocotylization in *E. dofleini* on the fourth right and fourth left arms.



FIG. 3. Reproductive organs of R4L4. White band on the left side is a developing spermatophore.

TABLE 2. Comparison of external attributes of R4L4 to means of similarly sized conspecifics*. Arm length and number of suckers for R4L4 was calculated using only intact standard arms.

	Arm length (cm) ± SE	Number of suckers ± SE	Total weight (kg) ± SE	Gonad weight (g) ± SE	Stomach weight (g) ± SE	Number of sper- matophores ± SE
R4L4	135 ± 1	230 ± 9	14.1	896	100	0
<i>E. dofleini</i> *	139 ± 1	245 ± 8	14.1 ± 0.1	932 ± 37	304 ± 48	4.0 ± 1.3

*13 to 15 kg in total weight (n = 21)

length, and number of suckers (Table 1). This contrasts with a previous report in which third right hectocotylized arms were shorter in total length, had more rows of suckers, and had a larger ligula than supernumerary hectocotylized arms (Palacio, 1973). As compared to other cases of double hectocotylization, the close similarity in the two hectocotylized arms of R4L4 suggests that anatomical anomalies may not be expressed similarly across all species or individuals.

Similarities in gonadal development and ligula length suggest that R4L4 was at a similar reproductive state and was equally successful in foraging for prey when compared to similar sized conspecifics. Though individual measurements of testes, vas deferens, seminal vesicle, prostate, Needham’s sac and genital bag were not taken, it appeared that spermatozoa were being processed into spermatophores in the seminal vesicle which was a sign of a maturing individual (Fig. 3). Though R4L4 did not have any fully formed spermatophores present, three of ten other *E. dofleini* examined also did not have spermatophores, suggesting that this level of reproductive maturity is within the normal range for similarly sized conspecific males. Furthermore, the standard arm length and number of suckers of R4L4 was similar to the other males, suggesting that double hectocotylization did not hinder the growth or number of suckers on typical arms (Table 2).

This is the first account of double hectocotylization for *E. dofleini* and double hectocotylization not involving the typical third right or left arm in any cephalopod species. It is possible that arm anomalies such as this are a regular occurrence, but due to a scarcity of research effort in many species, this is the first time this type of anomaly has been documented.

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RICKETTSIA-ASSOCIATED MORTALITY OF THE YELLOW CLAM
MESODESMA MACTROIDES (BIVALVIA: MESODESMATIDAE)
IN SOUTHERN BRAZIL

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INTRODUCTION

The yellow clam *Mesodesma mactroides* (Deshayes, 1854) is an intertidal sandy beach bivalve that is distributed from the southeast of Brazil (Ilha Grande, Rio de Janeiro State, 23°S) to Argentina (Isla del Jabalí, Buenos Aires Province, 41°S) (Rios, 2009). Historically, the yellow clam had been considered an important economic resource (Coscarón, 1959). However, yellow clam populations collapsed as a result of overfishing that was associated with repeated mass mortalities in Brazil, Uruguay and Argentina in the mid-1990s (Odebrecht et al., 1995; Fiori & Cazzaniga, 1999; Cremonte & Figueiras, 2004). Presently, *M. mactroides* is identified as a threatened species with a critically endangered status (Fiori & Cazzaniga, 1999).

As more knowledge about the factors that can influence the population sizes of sea animals have become available, it has become clear that disease can drastically affect abundance (Sindermann, 1990). Major disease-causing agents of marine bivalves include viruses, prokaryotes, fungi, protists, and invertebrates, such as Digenea, Annelida and Copepoda (Kinne, 1983). According to Lauckner (1983), prokaryotes belonging to Rickettsiales are capable of causing disease and possibly death in marine bivalves from natural beds around the world and, both juveniles and larvae of bivalves are more susceptible to diseases caused by prokaryotes infections (Elston, 1999). Rickettsiae are small, pleomorphic coccobacilli considered to occupy a special taxonomic niche between bacteria and viruses (Lauckner, 1983). The first rickettsia-like organisms detected in molluscs were found in *Mya arenaria* from Chesapeake Bay (Harshbarger et al., 1977). According to Harshbarger et al. (1977), the

ribosome-rich, undulating rods, measuring 300 x 2,000 nm occurred in roundish inclusions, up to 100 µm in diameter and usually located singly in the epithelial-cell cytoplasm and lumen of digestive diverticula.

Although the yellow clam has important economic value, few histopathological studies of this species have been completed. For example, Cremonte & Figueras (2004) reported *Trichodina* sp., gregarins, coccidians and Turbellaria in yellow clams from the Argentinean coast, and Brusa et al. (2006) discovered a new species of Turbellaria, *Paravortex mesodesma*, in the intestinal lumen of *M. mactroides* from the Uruguayan coast. However, the parasites identified in these studies could not be responsible for yellow clam mortality, and there is little known about the pathological conditions of *M. mactroides* populations in Brazil.

Despite the increasing concern about diseases in edible bivalves on the Brazilian coast, particularly in Santa Catarina State in southern Brazil (da Silva et al., 2002, 2011; Sabry et al., 2011) and the northeastern states of Brazil (Azevedo et al., 2005; Sabry et al., 2007; Boehs et al., 2010), the occurrence and impact of bivalve diseases remain poorly studied. Furthermore, mortalities can occur without notice because a systematic surveillance programme is not currently in place (da Silva et al., 2011). Prior to this study, no histopathological research on the diseases of bivalves had been completed in the State of Rio Grande do Sul.

The programme to monitor the health status of *M. mactroides* began in 2011. The study area included Cassino Beach, which lies between Rio Grande City and Barra do Chuí on the south shore of Rio Grande do Sul State in southern Brazil. In October 2011 (austral spring), the local recruitment period of *M. mactroides*

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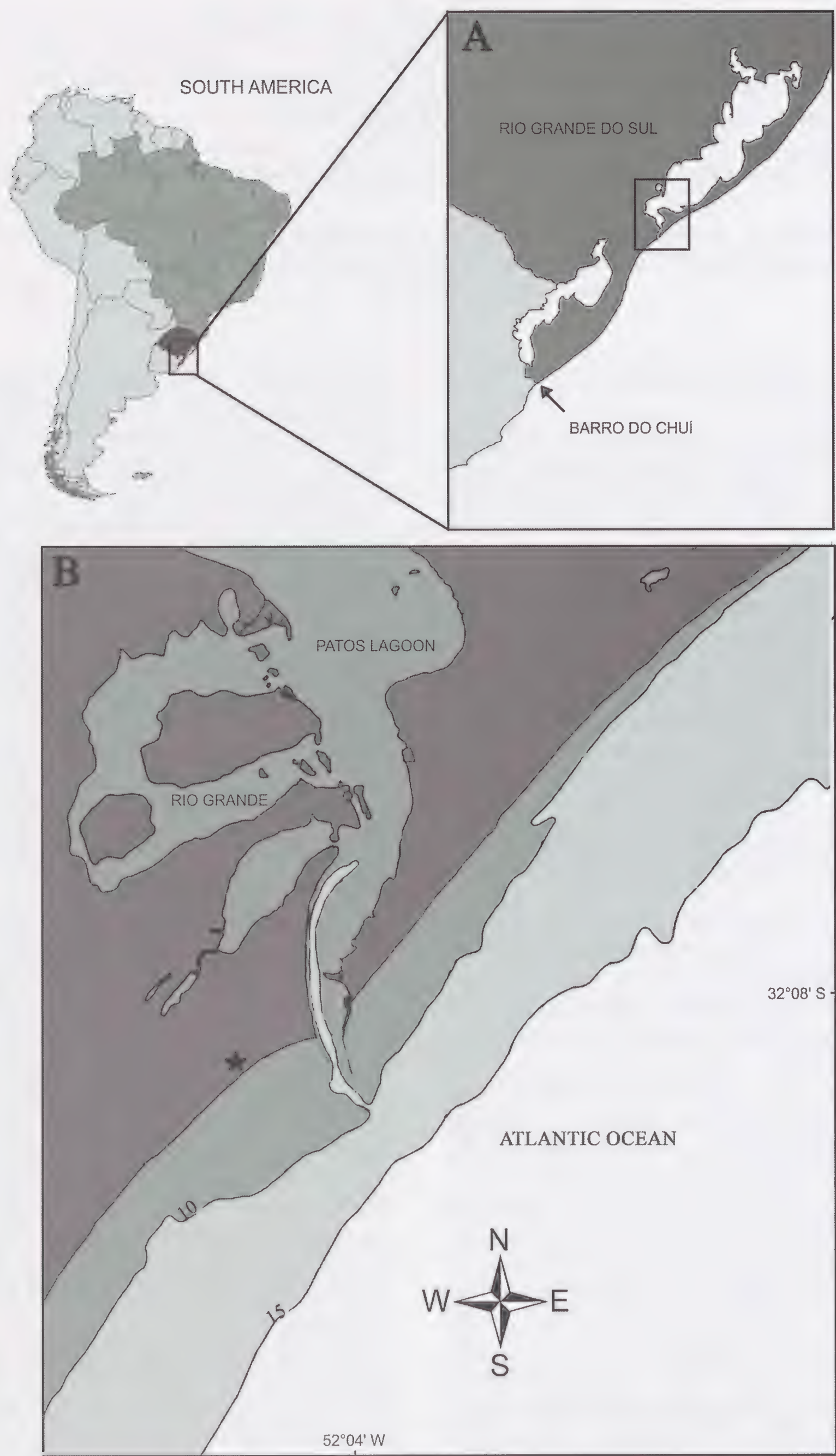


FIG. 1. Map of South America depicting the state of Rio Grande do Sul in southern Brazil. The star indicates the sampling location of moribund *Mesodesma mactroides* juveniles in Cassino Beach.

(Bergonci & Thomé, 2008), a large number of empty juvenile shells and moribund juveniles unable to burrow in the sand were observed at Cassino Beach. Initially it was thought that temperature or salinity could be responsible for the mortalities. However, abnormal fluctuations of these parameters were not observed (the temperature and salinity at the sampling time were 20°C and 30‰, respectively).

The objective of the present study was to determine the cause of the mortality event that affected juveniles of the yellow clam *M. mactroides* using histological assays for the detection of potential pathogens.

MATERIALS AND METHODS

Thirty moribund yellow clam juveniles (shell height ranging from 25 to 35 mm, mean = 29.35 ± 2.94 mm) were manually collected in the intertidal zone of Cassino Beach in southern Brazil (32°12'S, 52°11'W) (Fig. 1) in October 2011. The samples were transported to the laboratory as quickly as possible, and the soft parts of the moribund clams were carefully removed from their shells. They were fixed in Davidson's solution (Shaw & Battle, 1957) for one day and then stored in 70% ethanol.

Twenty whole individuals were processed with automated equipment (Lupe Tec, model PT 05, Brazil) and embedded in Paraplast®. Sections (3µm) were cut on a microtome (Lupe Tec, model MRP03, Brazil) and stained with haematoxylin and eosin (HE).

For transmission electron microscopy (TEM), small fragments of the intestines of ten clams were post-fixed in 2.5% glutaraldehyde and 1% OsO₄ in cacodylate buffer (0.05 M) (pH 7.4). The epithelial cells of the intestine were then dehydrated in ethanol and embedded in Epon 812 resin. Ultrathin sections were stained with uranyl acetate and lead citrate. Finally, the samples were examined with a microscope (JEOL, model 100S, Japan).

RESULTS

In 100% of the clams analysed, light microscopy revealed the accumulation of basophilic material infecting the epithelial cells of the intestine and the gills that corresponded to *Rickettsia* colonies (Fig. 2). No other parasites were recorded in the studied specimens.

Transmission electron microscopy of the infected midgut revealed varying numbers of rickettsiae in the lumen. Some were free in the lumen while others were intimately associated with the plasma membrane of the intestinal epithelial cells. In thin sections, the microorganisms were observed as circular shapes, approximately 0.2 to 0.6 µm wide and up to 1.6 µm in length. The limiting boundary was formed by two clearly defined structures: the rickettsial cell wall, which surrounds the whole microorganism, and the underlying plasma membrane, which limits the cytoplasm. Sections that were taken perpendicular to the cell wall and plasmalemma revealed the total thickness of the rickettsial interspace to be approximately 200 to 250 Å. The cell wall was approximately 90 Å thick, and the plasma membrane thickness was approximately 75 Å (Fig. 3B).

DISCUSSION

The present work investigated the cause of juveniles yellow clam (*Mesodesma mactroides*) mortality event that occurred in October of 2011 at an intertidal bed in Cassino Beach, State of Rio Grande do Sul, southern Brazil. Herein, no adults of yellow clam were recorded in the sampling area. According to Sindermann (1990), infectious diseases are common suspects in shellfish mortalities. In this study, we provide the first report of prokaryotes of the genus *Rickettsia* in the gill and intestinal epithelial cells of *M. mactroides*.

The order of Rickettsiales pertains to the Alphaproteobacteria class and is composed of microorganisms with an obligate intracellular and parasitic lifestyle (Dumler & Walker, 2005). This group of prokaryotes is best known for its medically important genus *Rickettsia*, whose species can cause severe and occasionally fatal diseases in man (Parola & Raoult, 2001). The ultrastructural characteristics of rickettsiae were clearly recorded in the present study. In thin sections, the microorganisms are seen as circular or rod-shaped profiles. The limiting boundary is formed by two clearly defined structures: the rickettsial cell wall, which surrounds the whole microorganism, and the underlying plasma membrane, which limits the cytoplasm. The contours of the rickettsiae are generally smooth, with few irregularities. Some specimens show wrinkled or bulbous outpocketings of the cell wall. The plasma-

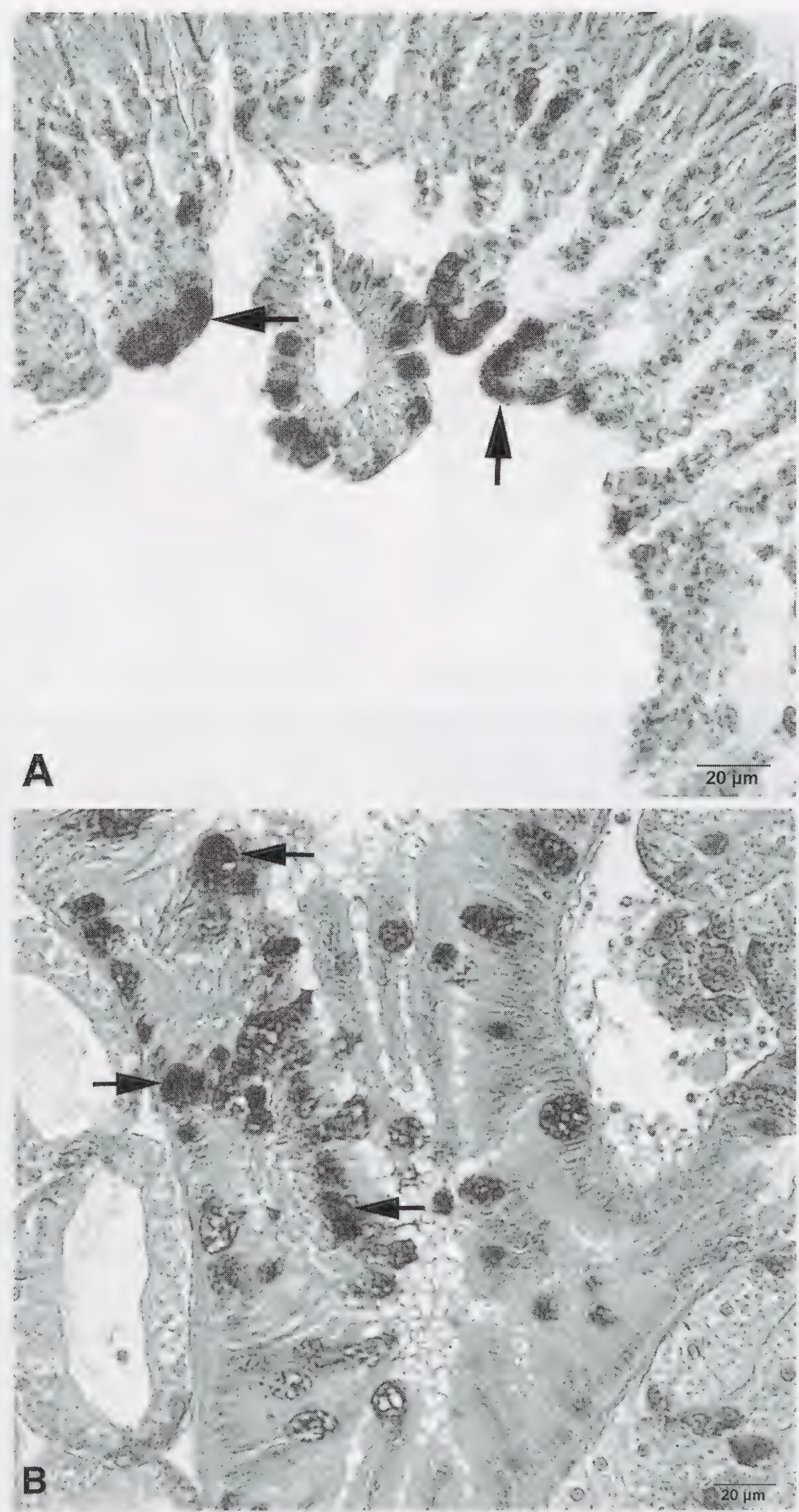


FIG. 2. *Mesodesma mactroides*. FIG. 2A: Gill of showing basophilic inclusions (arrows). Hematoxilin-Eosin staining; FIG. 2B: Intestinal epithelium with basophilic inclusions. Hematoxilin-Eosin staining.

lemma has occasional vesicular inpocketings, these invaginations resemble the pinocytotic vesicles or vacuoles seen in animal cells. Their contents are of low electron density and are continuous with the structure less clear space between the plasma membrane and the cell wall. At the site of these invaginations, the

cell wall is uninterrupted and does not parallel the infolded plasmalemma (Robertson, 1959; Cheville, 2009)
The first record of rickettsiae in bivalves was in 1977 in the soft shell clam, *Mya arenaria* from Chesapeake Bay, U.S.A. (Harshbarger et al., 1977). Since then, rickettsiae have been

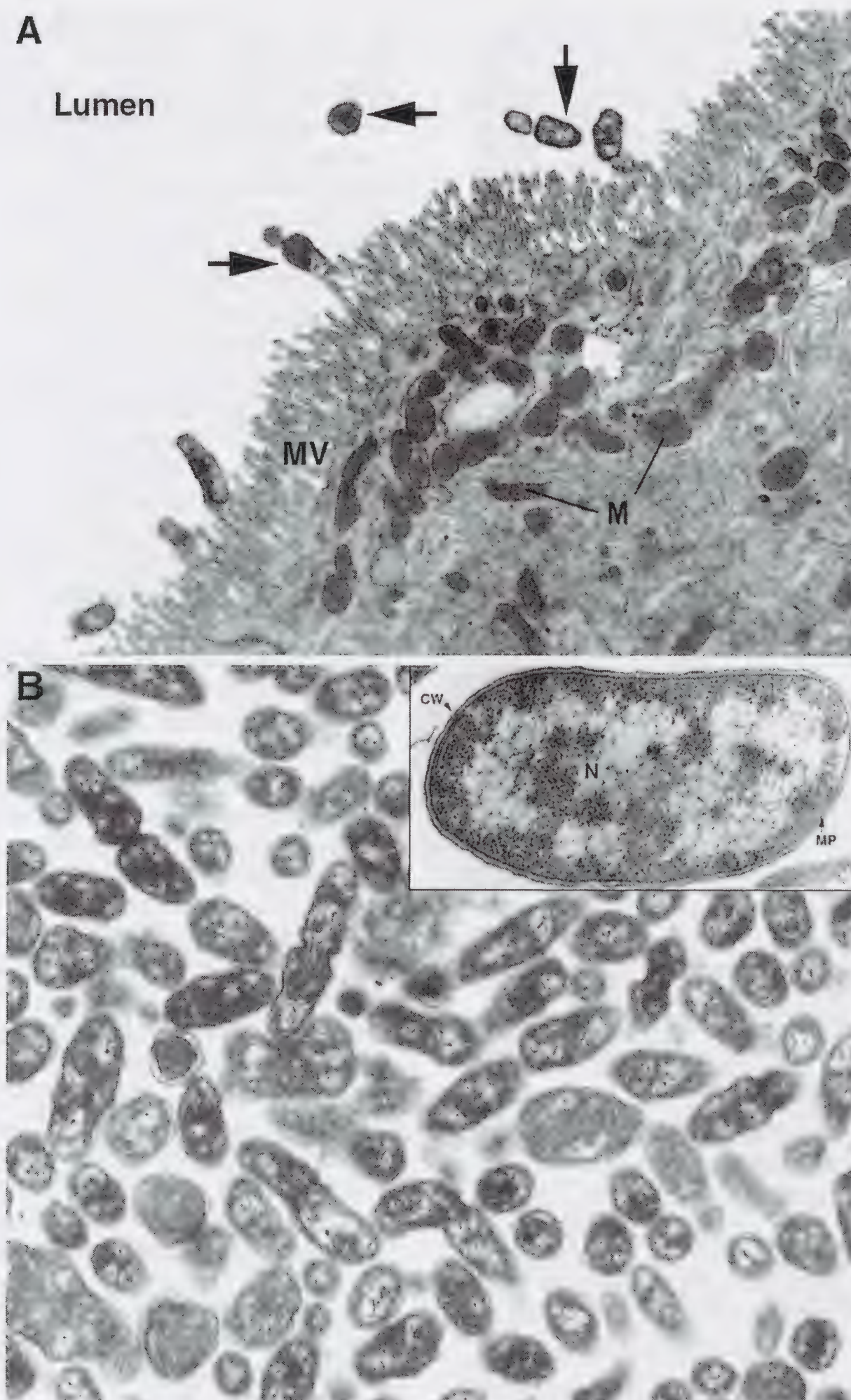


FIG. 3. Electron micrographs of *Mesodesma mactroides* and *Rickettsia*. FIG. 3A: Intestinal epithelium of *Mesodesma mactroides*. The lumen contains several rickettsiae (arrows); some are in close association with the microvilli (MV). The dense mitochondria in the epithelial cells have a different internal structure than the extracellular microorganisms (M). X 9,500; FIG. 3B: Central portion of a colony of *Rickettsia*. Circular profiles represent transverse sections, and elongate forms are sectioned longitudinally. Note the constriction furrows across the centres of several rickettsiae. With the exception of several pleomorphic forms, the colony is remarkably homogeneous. X 24,000. Insert: *Rickettsia* cells from the intestine of an infected *Mesodesma mactroides*. Both cell wall (CW) and plasma membrane (MP) are trilaminar when viewed on edge. The nuclear component (N) is represented as areas with fine filaments. X 125,000.

reported in several bivalve species, and they predominantly inhabit in the epithelia of the host's intestines and gills (Buchanan, 1978; Elston & Peacock, 1984; Mialhe et al., 1987; Fries & Grant, 1991). Members of the order Rickettsiales have previously been associated with mortalities of natural populations of different invertebrate groups (Sparks, 1985).

Some studies claim that *Rickettsia* infections cause little harm to bivalves as too few cells become infected (Harshbarger et al., 1977; Bower et al., 1994; Cremonte et al., 2005; Boehs et al., 2010). Indeed, when *Rickettsia* infection occurs in high intensity, it has been associated with significant mortalities in bivalve populations (Buchanan, 1978; Lauckner, 1983; Elston, 1986; Le Gall et al., 1988; Norton et al., 1993; Villalba et al., 1999). Bivalve larvae and juveniles are more vulnerable to prokaryote infections than adults as stated by Kinne (1983) and Elston (1999). In other invertebrates and vertebrates, rickettsiae that invade the intestinal epithelial cells and produce an alteration in nutrient absorption (Walker et al., 2003). It may also happen in *Mesodesma mactroides*.

The first description of a Rickettsiales-like organism (RLO) in the Brazilian aquatic fauna was recorded by Azevedo et al. (2005). They found RLOs infecting the gill epithelium of the mangrove oyster, *Crassostrea rhizophorae*, in the State of Piauí in northeastern Brazil. They suggested that RLOs could be associated with mortality of the parasitised oyster due to the disappearance of the apical cilia with concomitant lysis of the parasitised epithelial cells of the gill. Da Silva et al. (2011), and Sabry et al. (2011) found RLOs in very low prevalence in epithelial cells of the digestive glands of oysters (*Crassostrea gigas* and *C. rhizophorae*) from Santa Catarina State in southern Brazil, and those cells did not sustain serious damage.

This is the first report of *Rickettsia* infection among *Mesodesma mactroides* and the first histopathological survey of diseases of marine bivalves from Rio Grande do Sul State. We observed that 100% of the bivalve specimens analysed were infected with *Rickettsia*. Furthermore, the specimens were moribund at the time of collection, and upon returning to the sampling site for observations, it was noted that all juveniles were dead. Therefore, our data suggests that *Rickettsia* contributed to the mortality event of *M. mactroides* reported in Brazil in 2011.

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VALIDITY OF *HIATULA* MODEER, 1793 (BIVALVIA: PSAMMOBIIDAE)

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INTRODUCTION

In 1793, Adolph Modeer, a Swedish naturalist, proposed *Hiatula* as a bivalve genus-group name in the introduction to his study of bivalve mollusks. However, the validity of *Hiatula* Modeer, 1793, has been open to debate, because of the ambiguous original description lacking a type designation, the polyphyletic originally included nominal species (ICZN Art. 67.2), and the existence of probable senior and junior homonyms, as discussed by several authors (e.g., Dall, 1900; Winckworth, 1935; Rehder, 1967; Roth & Guruswani-Naidu, 1974; Beu & Maxwell, 1990; Willan, 1993). I herein re-evaluate the validity of this genus-group name in light of the philology and in accordance with the provision of *International Code of Zoological Nomenclature* (ICZN) (International Commission on Zoological Nomenclature, 1999).

Modeer (1793: 178–179) described *Hiatula* in Latin as follows (Fig. 1):

“*HIATULA* (Gapmussla): *Testa* aequalis oblonga, utrinque hians. *Cardo* valvae unius dente depresso, patulo: inserto aut vacuo; alterius subbidentato, plerisque scrobiculo intermedio, excavato. *Dentibus* lateralibus aut nullis, aut vulvali tantum, raro anali obsoleto. *Animal* Pallio lobato, pede disticho subfalcato, *Tentaculis* lanceolato-subtriquetris; *Trachea* longissima, simplici.”

Translation: [*HIATULA* (Gape clam): Shell equally oblong, gaping at both ends; Hinge of valves with a single, depressed, broad tooth: or insert in a socket; others weakly bidentate; with several, excavate intermediate pits. Lateral teeth either none or only in small valves; posterior rarely obsoleted. Pallial animal lobate; pedal distichous, subfalcate; gills lanceolate-subtriquetral; siphon very long, simple.

Modeer (1793: 182–183) also made the following remarks in Swedish:

“Ifrån SOLEN är HIATULA, både til Skäl och Kräk, få åskildt [sic; åtskild] som natt och dag, hvarföre ock til den senare höra de som

förut hetat SOLEN diphos och Bullatus, MYA arenaria och truncata, MYTILUS Pholadis o. s. v.”

Translation: From *Solen*, *Hiatula* is distinguished both in the shell and animal, as night and day, and the wherefores the latter also refers to those previously called *Solen diphos* and *bullatus*, *Mya arenaria* and *truncata*, *Mytilus pholadis* etc.

On the basis of the above-quoted remarks, the following five species – Willan (1993) incorrectly noted them as “three species” – are the originally included nominal species: *Solen diphos* Linnaeus, 1771; *Solen bullatus* Linnaeus, 1758; *Mya arenaria* Linnaeus, 1758; *Mya truncata* Linnaeus, 1758; and *Mytilus pholadis* Linnaeus, 1771.

On the other hand, Blainville (1824: 350) proposed *Soletellina* with a sole example of the species, “La Soletelliné rostrée. *Soletellina radiata*.” Therefore, *Soletellina radiata* Blainville, 1824, is its type species by monotypy (ICZN Art. 68.3). It may be noted that the species name corresponding to the French name “la Soletelliné rostrée” would be “*Soletellina rostrata*”. The reference “E. m., 336, f. 1” was cited for this species. The abbreviation “E. m.” refers to the “*Encyclopédie méthodique*”, and the reference is to Lamarck (1798: pl. 336, fig. 1). However, the plate number was incorrect, because pl. 336 depicts conid gastropods only. The correct reference is Bruguière (1797: pl. 226, fig. 1), in which “*Solen rostratus*. Lamk. v. 456” [*Solen rostratus* Lamarck, 1818: 456] was illustrated (fig. expl.: Bory de Saint-Vincent, 1827: 149). As pointed out by previous authors, these species are synonymous with *Solen diphos* Linnaeus, 1771 (e.g., Willan, 1993).

Subsequently, H. Adams & A. Adams (1856: 392) redefined *Hiatula* Modeer, 1793, as a tellinoidean bivalve genus and treated *Soletellina* Blainville, 1824, as a junior synonym. Although they referred to 17 species as being members of this genus, no type species was designated. Thereafter, Stoliczka (1870: 114) designated *Solen diphos* Linnaeus, 1771, one

12. *HIATULA* (*Gapmusfla*): *Testa* æqualis oblonga, utrinque hians. *Cardo* valvæ unius dente depresso, patulo: infero aut vacuo; alterius subbidentato, plerisque scrobiculo intermedio, excavato. Dentibus lateralibus aut nullis, aut vulvali tantum, raro anali obsoleto.

Animal

Animal Pallio lobato, pede disticho subfalcato, Tentaculis lanceolato-subtriquetris; Trachea longissima, simplici.

FIG. 1. Original description of *Hiatula* Modeer, 1793 (reproduced from Modeer, 1793: 178–179; composite image of two pages).

of the originally included nominal species, as the type species. Thus, *Hiatula* Modeer, 1793, has the same type species as *Soletellina* Blainville, 1824. This view was supported by Bertin (1880), Tryon (1884), Zittel (1881–1885), Lamy (1914), and other authors.

Gill (1892), in contrast, noted that *Hiatula* Modeer, 1793, has several homonyms, including a senior homonym *Hiatula* Martini, 1774 (p. 114). Dall (1900: 857, 971) regarded *Hiatula* Modeer, 1793, as a junior synonym of *Mya* Linnaeus, 1758, and pointed out that *Hiatula* Modeer, 1793, was merely a revival of *Hiatula* Schröter, 1784 (p. 599). Winckworth (1935: 322) designated *Mya truncata* Linnaeus, 1758, as the type species of *Hiatula* Modeer, 1793, which would make this name the junior objective synonym of *Mya* Linnaeus, 1758. MacNeil (1965), Vokes (1967), and Keen (1969a, b) followed Winckworth's (1935) view. However, these authors overlooked Stoliczka's (1870) type designation.

In his taxonomic study of the family Psammobiidae in the Australian and New Zealand region, Willan (1993) argued for bringing a case to the ICZN for suppression of Stoliczka's (1870) type designation on grounds of: (1) nomenclatural stability; (2) the likelihood that Modeer's (1793) original description only corresponded to *Mya* Linnaeus, 1758; (3) homonymy with *Hiatula* Martini, 1774; and (4) incorrect type designations by Stoliczka (1870) for other psammobiid genera. Willan (1993) expected that the Plenary Powers of ICZN would leave Winckworth's (1935) designation

as the valid one and *Hiatula* Modeer, 1793, would thereby become an objective synonym of *Mya* Linnaeus, 1758. Thus, he tentatively used *Soletellina* Blainville, 1824, as a valid name. Although such a petition to ICZN was not made, some authors have preferred to use *Soletellina* Blainville, 1824, instead of *Hiatula* Modeer, 1793 (e.g., Willan, 1998; Higo et al., 1999; Matsukuma, 2000; Min, 2004; Wang, 2004; Huber, 2010; Willan & Tagaro, 2011; Robin, 2011; Amano & Ogiyara, 2012).

However, the type fixation by Stoliczka (1870) for *Hiatula* Modeer, 1793, is valid under ICZN Arts. 67.2, 67.5, 69.1, and 69.2; and thus the type designation by Winckworth (1935) is invalid under ICZN Art. 69.1.1. The correspondence between the original description and the morphologic characters of the type species is irrelevant to the validity of the subsequent type designation (under ICZN Art. 69.1.1). As pointed out by Rehder (1967), *Hiatula* Martini, 1774, is unavailable, because it was published in a non-binominal work (ICZN Art. 11.4). In addition, *Hiatula* Schröter, 1784, is unavailable, having been introduced as a synonym of *Mya* Linnaeus, 1758, without nominal species. Subsequently, Dall (1900: 857, 971) treated it as an available name, namely, a junior synonym of *Mya* Linnaeus, 1758, and a senior synonym of *Hiatula* Modeer, 1793. However, it cannot be treated either as the name of a taxon or as a senior homonym, because it does not fulfill the requirement of ICZN Art. 11.6.1. Moreover, in the last 50 years, *Hiatula* Modeer, 1793, has been broadly accepted as a valid name (e.g.,

Rehder, 1967; Habe & Kosuge, 1967; Kuroda et al., 1971; Oyama, 1973; Roth & Guruswami-Naidu, 1974; Itoigawa et al., 1974, 1981, 1982; Habe, 1977, 1981; Kilburn, 1977; Ogasawara & Noda, 1978; Wu, 1980; Abbott & Dance, 1982; Shibata & Ina, 1983; Okutani et al., 1989; Beu & Maxwell, 1990; Oliver, 1992; Ogasawara & Nagasawa, 1992; Lee, 1992; S. Morris & N. Morris, 1993; Bernard et al., 1993; Matsubara, 1995, 2011; Nakagawa, 1998; Wang, 1998; Shibata & Ichihara, 2006; Sasaki & Matsubara, 2010). These conditions do not fulfill the requirements in ICZN Art. 23.9, and reversal of precedence cannot be applied to this case.

In conclusion, *Hiatula* Modeer, 1793, is a valid name, with *Solen diphos* Linnaeus, 1771, as its type species (by the subsequent designation of Stoliczka, 1870), and it cannot be considered a junior synonym of *Mya* Linnaeus, 1758. The two senior homonyms, *Hiatula* Martini, 1774, and *Hiatula* Schröter, 1784, are unavailable. *Soletellina* Blainville, 1824, which has the same type as *Hiatula* Modeer, 1793, is a junior objective synonym, and henceforth, should not be used as a valid name.

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RECLASSIFICATION OF *PANOPEA GENEROSA* VAR. *TAENIATA* DALL, 1918, A FOSSIL MORPHOTYPE OF *P. GLOBOSA* DALL, 1898

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INTRODUCTION

Two extant species of geoduck clams occur on the coast of the Baja California Peninsula. *Panopea generosa* Gould, 1850, occurs from southern Alaska, U.S.A., to Punta Eugenia on the Pacific coast of the Baja California Peninsula, and *P. globosa* Dall, 1898, which was historically considered endemic to the Gulf of California. Despite their geographic proximity, *P. generosa* and *P. globosa* are not phylogenetically close (Rocha-Olivares et al., 2010). In addition, based on morphological and genetic similarity, it has been shown that *P. globosa* occurs outside the Gulf of California on the Pacific coast of the southern Baja California Peninsula in Bahía Magdalena (Leyva-Valencia, 2012; Leyva-Valencia et al., 2012).

The nomenclature of the Pacific coast species of this genus has been controversial. For example, Vadopalas et al. (2010) showed that despite distinct morphological differences between the holotypes of *P. generosa* and *P. abrupta* (Conrad, 1849), they were mistakenly synonymized in 1983. Dall first described *Panopea globosa* as a variety of *P. generosa* from a shell collected at San Felipe in the northern Gulf of California; he also described the fossil *P. solida*, from San Francisco, California (Dall, 1898). Twenty years later, Dall described a new variety of *P. generosa* – *P. generosa* var. *taeniata*, Dall, 1918 – from a fossil collected in Pleistocene marine sediments on Isla Magdalena (Fig. 1), a site directly adjacent to extant aggregations of what are now recognized as *P. globosa* in Bahía Magdalena, Baja California Sur.

The fossil record of *P. globosa* available in the paleontology collection of the San Diego Natural History Museum (SDNHM) indicates that this species was sympatric with *P. generosa* from the late Miocene to the Pleistocene in the predecessor of the Salton Sea in southern California, and fossils of both species have

been found in Puertecitos in the upper Gulf of California (Judith Smith, Smithsonian Institution, National Museum of Natural History, 2011, pers. comm.). Although the Baja California fossil record is not well characterized, *P. globosa* was present during the Pliocene and Pleistocene in the middle, southern and western coast of the Peninsula (Scott Rugh, San Diego Natural History Museum, 2010, pers. comm.; Gerardo Barba, Universidad Autónoma de Baja California Sur, 2010, pers. comm.; Judith Smith, 2011, pers. comm.), in accord with the distribution of extant aggregations.

The goal of this study was to determine whether *P. taeniata* is, as Dall described, a variety of *P. generosa*, or a fossil of *P. globosa*. The latter would suggest that the *P. globosa* distribution extended out of the Gulf of California prior to the last glacial period.

METHODS

Shell shape analysis was performed using a geometric morphometric method, as previously used to discriminate between *Panopea generosa* and *P. globosa* (Leyva-Valencia et al., 2012). Digitized images were captured at 300 dpi in JPG format (Hewlett-Packard ScanJet 2200c) of the holotypes *P. generosa* (USNM 5844), *P. globosa* (USNM 74884), *P. taeniata* (USNM 372847) and *P. solida* (USNM 3480), as well as 180 specimens of *P. globosa* from Bahía Magdalena (n = 50), San Felipe (n = 50), Puerto Peñasco (n = 50) and Guaymas (n = 30); and 204 of *P. generosa* from Alaska (n = 11), Washington (n = 55), California (n = 54) and Baja California (n = 84).

All images were imported to tpsUtil 1.44 (Rohlf, 2009). We selected outline landmarks using the fan-based method (MakeFan6 software; Sheets et al., 2006). We also selected three internal anatomical landmarks and 48

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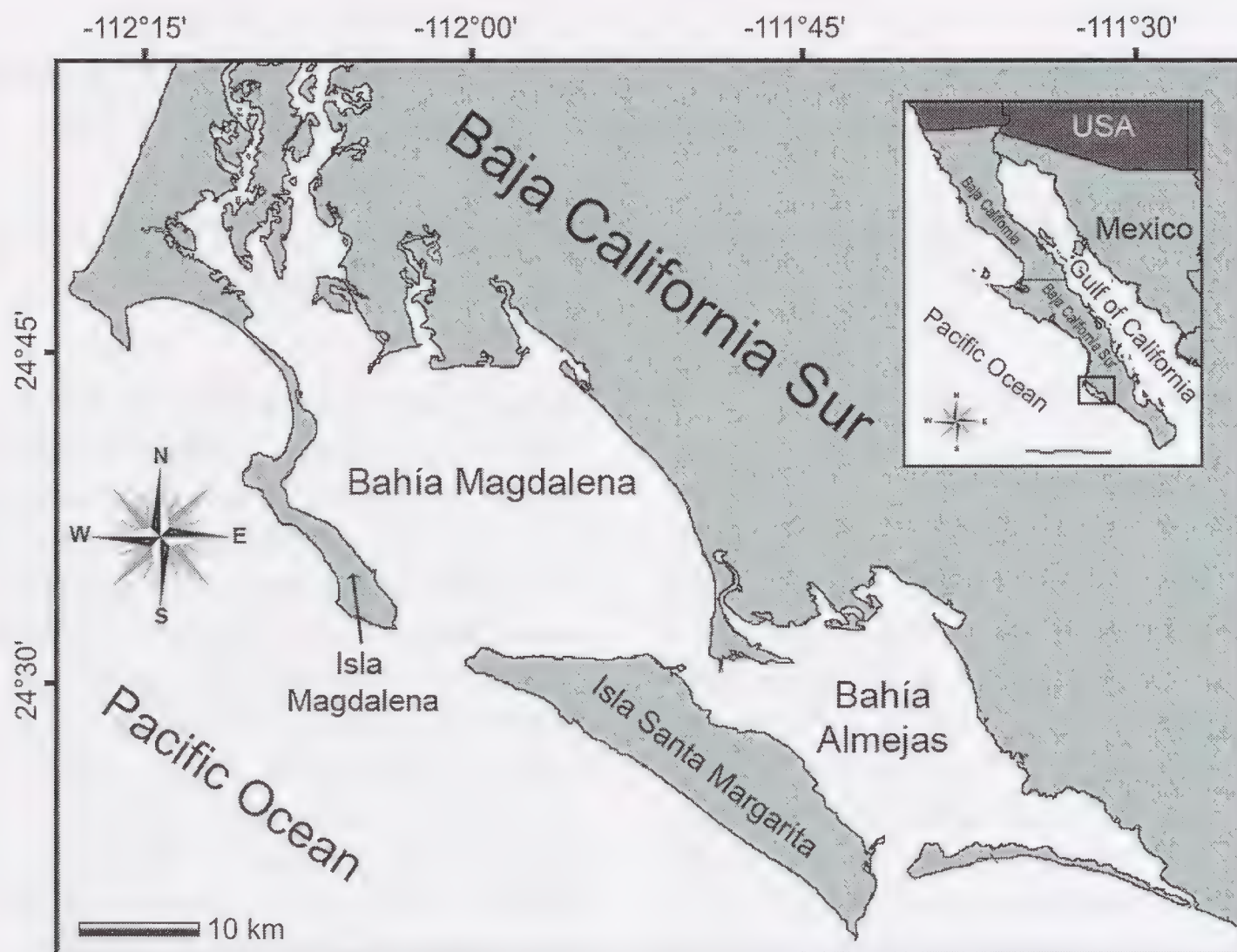


FIG. 1. Location of Isla Magdalena, collection site of *Panopea generosa* var. *taeniata* holotype.

semi-landmarks to generate two-dimensional Cartesian coordinates using tpsDig 2.12 (Rohlf, 2008). To minimize least-squares differences between landmark alignments and produce Procrustes shape coordinates, we used IMP: CoordGen6f software (Sheets et al., 2006). To accurately slide semi-landmarks along a contour to minimize the bending energy of deformation for a given specimen, we used IMP: SemiLand6 (Sheets et al., 2006). To characterize the shell outline variation between groups, we used canonical variates analysis and multivariate analysis of variance (CVA-MANOVA) in CVAGen6m (Sheets et al., 2006).

RESULTS AND DISCUSSION

The analyses of outline and internal landmarks revealed only one highly discriminant canonic variate separating *Panopea generosa* from *P. globosa*. *Panopea taeniata* was included within the *P. globosa* group, whereas *P. solida* was included within the *P. generosa* group (Fig. 2).

The shell morphology of *P. taeniata* agrees with the taxonomic characteristics of *P. globosa*. Both species have a deeper pallial sinus than *P. generosa*, and the nymph length of *P. taeniata* is shorter than in *P. generosa*. The *P.*

taeniata valve contour also shows the same inflated anterior region and narrow posterior end as *P. globosa* (Fig. 3A). On the other hand, the *P. solida* valve agrees with the general phenotype of *P. generosa*. *Panopea solida* and *P. generosa* holotypes show a hard and large nymph, with a similar pallial sinus morphology, dorsal and ventral margins with parallel growth to the shell length, a rounded anterior region and a wide posterior region (Fig. 3B). A recent morphometric study between *P. generosa* and *P. globosa* revealed great interspecific differences on anteroventral region, posterior end, pallial sinus depth and nymph length (Leyva-Valencia et al., 2012). Our results show that *P. taeniata* is not a fossil subspecies of *P. generosa*, and is hereby reclassified as a fossil morphotype of *P. globosa*.

The identification of *Panopea globosa* fossils from Isla Magdalena suggests that the extant *P. globosa* aggregation in Bahía Magdalena is ancient, at least 125,000 years old. The origin of this population is unknown, although the Pliocene to Pleistocene fossil record along of the Gulf of California and San José del Cabo Basin agree with the hypothetical migration pattern from the Gulf of California to the Bahía Magdalena area (Judith Smith, Smithsonian Institution, National Museum of Natural History, 2011, pers. comm.). Bahía Magdalena

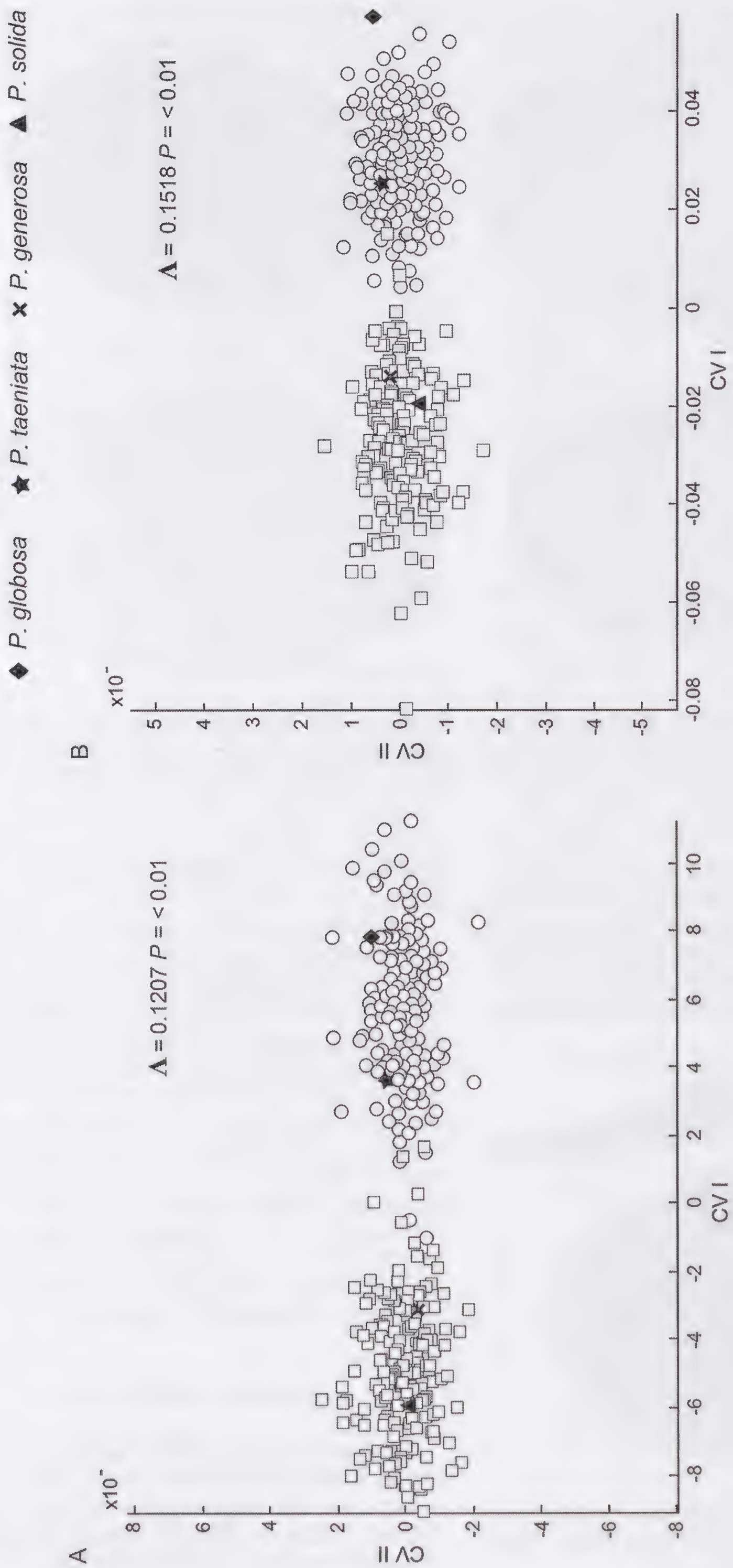


FIG. 2. CVA analyses of *Panopea generosa* (□) and *P. globosa* (○). A: Discriminate contour landmarks among *P. globosa*, *P. taeniata*, *P. generosa* and *P. solida* holotypes; B: Discriminate internal landmarks among *P. globosa*, *P. taeniata*, *P. generosa* and *P. solida* holotypes.

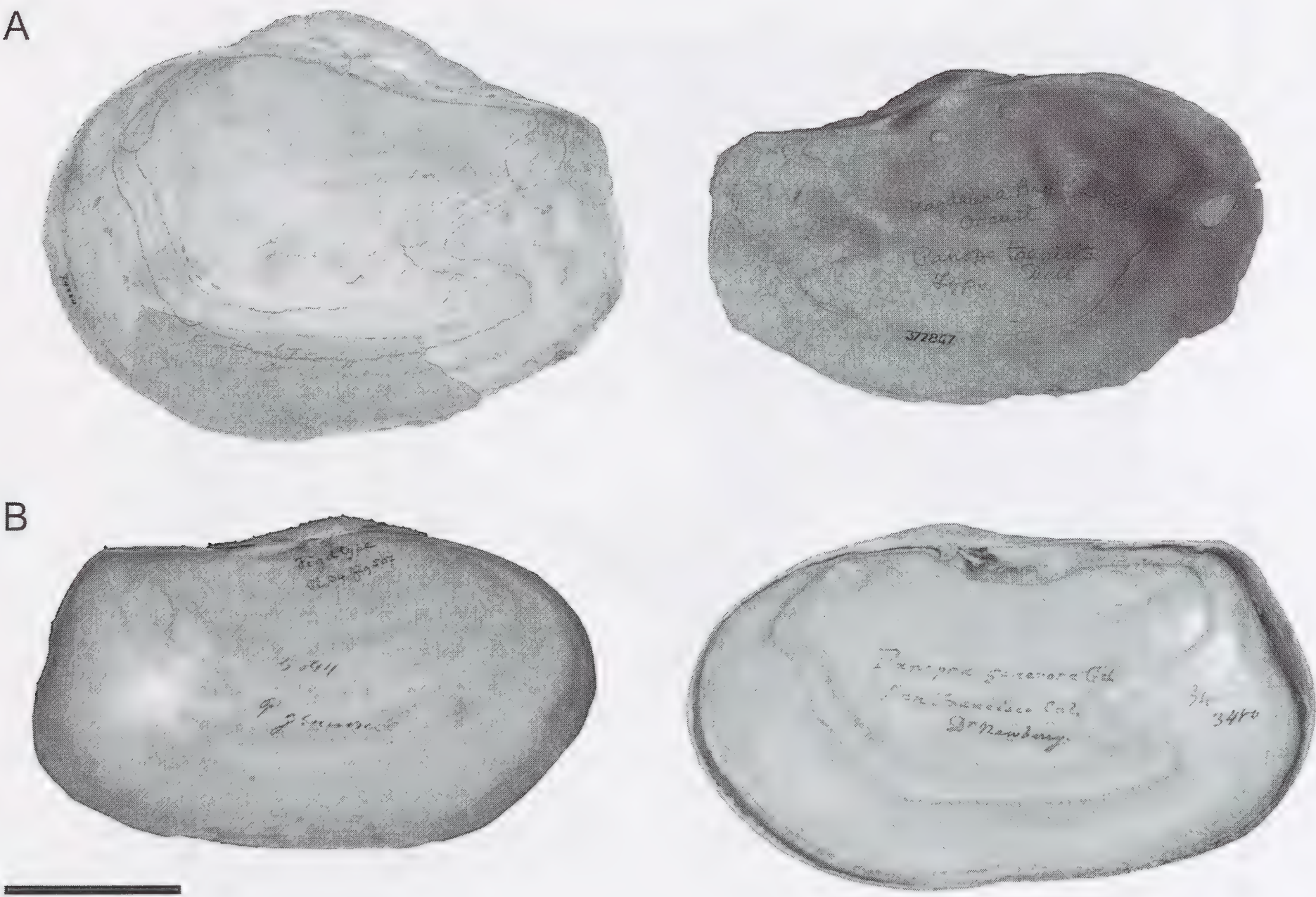


FIG. 3. Contour similarities of *Panopea* spp. A: Contour similarities between *Panopea globosa* (left) and *P. taeniata* (right). Images courtesy of Judith Smith, USNM 2012; B: Contour similarities between *P. generosa* (left) and *P. solida* (right). Images courtesy of Yolanda Villacampa, USNM 2010. Scale bar = 5 cm.

and Bahía Almejas are tectonic structural lagoons (Lankford, 1977) formed by northwest-southeast faulting along the Tosco-Abrejos fault zone around 20,000 years ago. Rising sea levels filled depressions, and the uplifted side of the fault gave rise to Isla Santa Margarita and

Isla Magdalena (Spencer & Normark, 1979; Blake et al., 1984).

Panopea taeniata fossils also have been recorded in Bahía Santa Ines, near Santa Rosalia in the central Gulf of California (Fig. 4; Durham, 1950; Libbey & Johnson, 1997). Although we cannot analyze this specimen using morphometric methods, the image permits morphologic traits to be distinguished, including a narrow posterior end, non-parallel dorsal and ventral margins, a wide anterior end as well as a short nymph. These traits are shared with *P. globosa*, and thus we conclude that this specimen was misidentified as a subspecies of *P. generosa*. In addition to the reclassification of the *P. taeniata* holotype, fossils such as that of Durham (1950) should be attributed to *P. globosa*.

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FIG. 4. Fossil of *P. generosa* var. *taeniata* from Bahía Santa Ines, Gulf of California. Image modified from Durham (1950).

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REPRODUCTIVE BIOLOGY OF NAIADS IN THE UPPER BLUE NILE

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INTRODUCTION

The freshwater mussels of the order Unionida (naiads) are unique because their larvae, which are brooded in marsupial demibranchs, are obligate parasites on fish hosts. Their evolution has been influenced by a long and complex history that ranges over the entire planet and extends over 200 million years (Watters, 2001). However, their systematic relationships (e.g., monophyletic versus polyphyletic origin, number of families and species etc.) are still heavily debated (e.g., Graf & Cummings, 2006, 2007; Hoeh et al., 2009; Whelan et al., 2011), particularly because of two different types of larvae, glochidium and lasidium.

The components of the naiad assemblage in the Nile have evolutionary ties to a wide range of taxa around the world. Three families occur in the Nile system, Unionidae, Iridinidae and Etheriidae (Graf & Cummings, 2007). Furthermore, the Nile system is the only area where palaeartic and Afro-tropical molluscs overlap. During the Quaternary cold phases, palaeartic species extended as far south as Lake Turkana. Now they are separated from Afro-tropical species by the Sahara and occur mainly in the coastal areas of northwest Africa. The only river crossing the Sahara from tropical Africa north to the Mediterranean Sea is the Nile, and palaeartic species still extend as far south as to its headwaters (Van Damme, 1984).

For most Nile species, data on reproductive traits would be necessary to detect their relation to other taxa around the world. In biogeographical respect, this naiad fauna is marked by:

- (a) a high rate of endemism to the Nile,
- (b) the southern extent of Palaeartic species, and
- (c) Afro-tropical species.

The three naiad species surveyed here – discussed by Van Damme, 1984, and Graf & Cummings, 2007 – are representatives of these three categories:

Unio abyssinicus (Martens, 1866), is a Blue Nile endemic of an otherwise mainly European genus. During the Pleistocene, it was widely

distributed in the Nile system up to northern Egypt, but it disappeared from most areas during the early or middle Holocene (10,000 – 5,000 B.C.), and it now occurs in Lake Tana and the upper Blue Nile (Van Damme, 1984; Graf & Cummings, 2007). The reproductive biology of this species has been unknown.

Unio mancus (Lamarck, 1819) (synonym: *U. elongatulus* Rossmäessler, 1835) is distributed around the Mediterranean Sea (Araujo et al., 2005). It is the common *Unio* of southern France, northeastern, central and southern Spain, Italy and parts of the Middle East (Nagel et al., 1998, Araujo et al., 2005). Two African subspecies are geographically isolated. According to Van Damme (1984), the one occurring in Lake Tana and the Blue Nile is considered *U. mancus dembeae* (Reeve, 1865). It is the only palaeartic naiad reaching the Nile (Graf & Cummings, 2007; Van Damme, 1984). Reproductive data are available for some populations of *U. mancus* on the Iberian Peninsula (Araujo et al., 2005) and Italy (Castagnolo, 1977), but not for *U. mancus dembeae*.

Etheria elliptica (Lamarck, 1807), the cementing Nile Oyster, is widespread in tropical Africa, where it is found in the basins of the Nile, Niger, Congo and even in Madagascar. The northern limits of its range are the Senegal and Niger Rivers and Lake Tana. The oldest fossils date from the Early Miocene; however, its occurrence in Madagascar suggests a greater phylogenetic age (Yonge, 1962; Van Damme, 1984). Its systematic position and its relationship to the other two cementing naiad species in South America and South India is unclear (Hoeh et al., 2009; Bogan & Hoeh, 2000), and little is known about its life history. In particular, data on its reproductive biology has not been available. Some subspecies or races have been described, which are very different in size and shell morphology (Yonge, 1962). My results indicate also different modes of reproduction. Therefore, in this paper I use just the generic name *Etheria*.

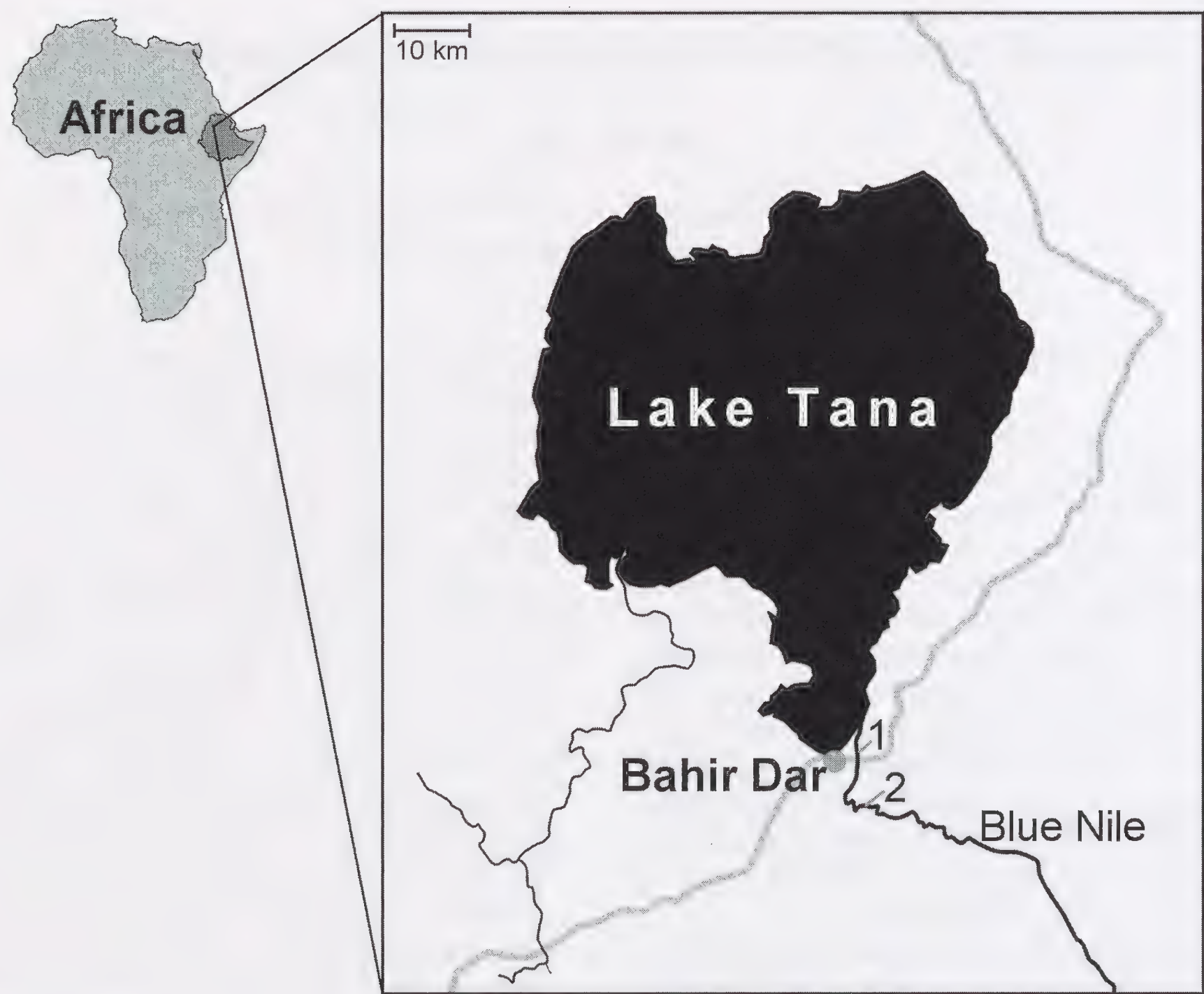


FIG. 1. The study area Lake Tana and the origin of the Blue Nile in the Ethiopian Highland; 1 = Sample site 2009, 2 = Sample site 2010.

METHODS

The investigations were conducted in Bahir Dar, Ethiopia, at the origin of the Blue Nile (Fig. 1), which is the outlet of Lake Tana at an altitude of approximately 1,700 m. Mussels were collected by fishermen, in the first study period (January – February 2009) from the Blue Nile close to Lake Tana, in the second study period (March 2010) approximately 10 km downstream of Bahir Dar (Fig. 1). During this second period, only *Etheria* was collected.

TABLE 1. Reproductive status of 46 *Unio abyssinicus* individuals in the upper Blue Nile (January 2009).

Shell length (mm)	30–40 40–50 50–60 > 60				sum
males	1	5	13	2	21
females		6	14	5	25
gravid		3	9	2	14

All specimens of *Unio* were preserved as a whole in 100% ethanol. In Germany, they were examined for gravidity, and sex was determined by making an incision in the visceral mass dorsal to the foot and analysing the smear under the microscope. Oocytes and spermatozoa could be easily recognized.

Etheria was dissected in Ethiopia, and the gills were examined using a binocular microscope for gravidity. Pieces of gravid gills were fixed in ethanol for further examination in Germany.

The measurements of egg or larval sizes were performed using an Zeiss Axioskope with the Optimas programme. Some glochidia were cleared with KOH to show the shell structures.

RESULTS

Unio abyssinicus

We collected 46 specimens. They were dioecious, with a nearly equal sex ratio (Table 1).

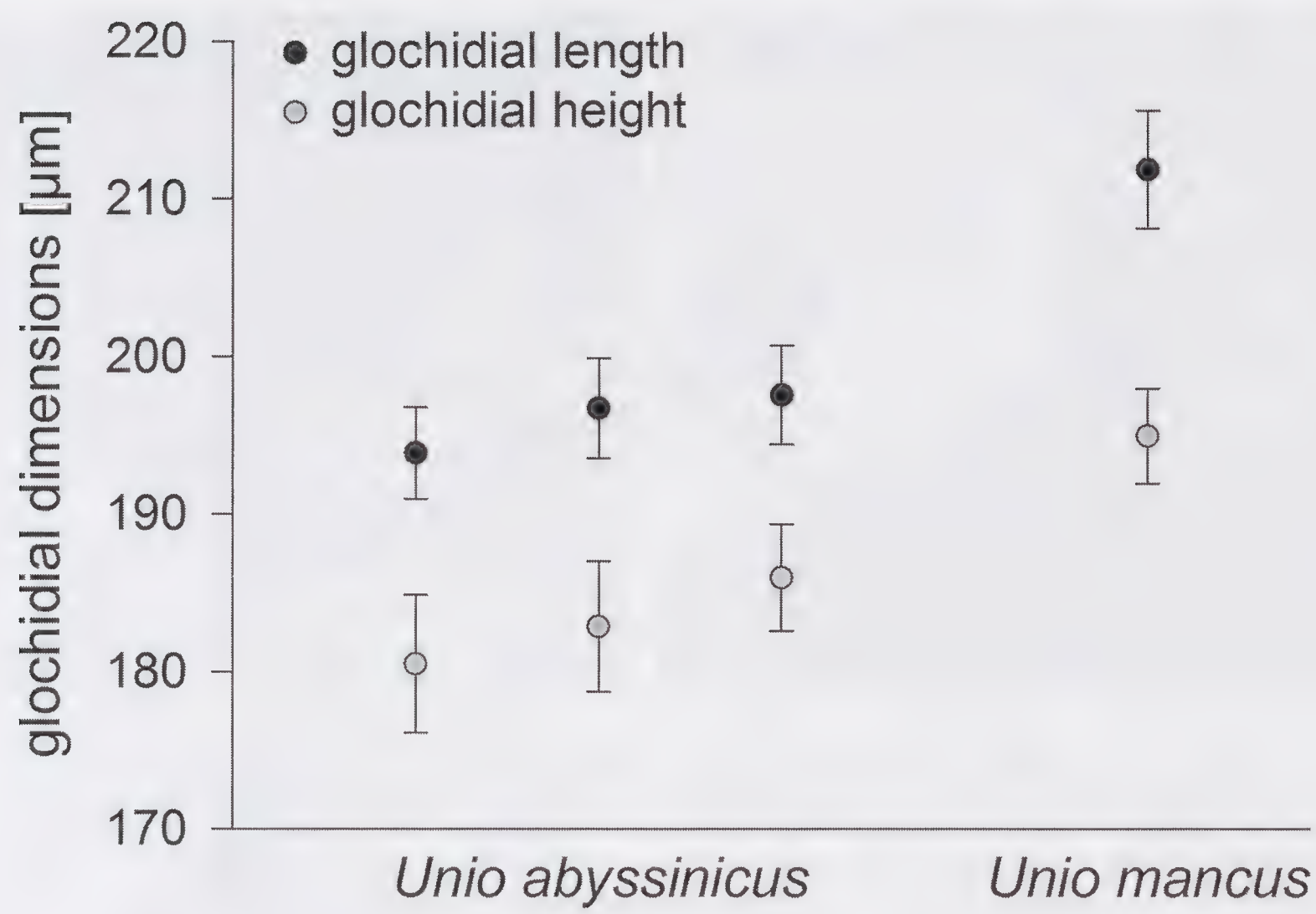
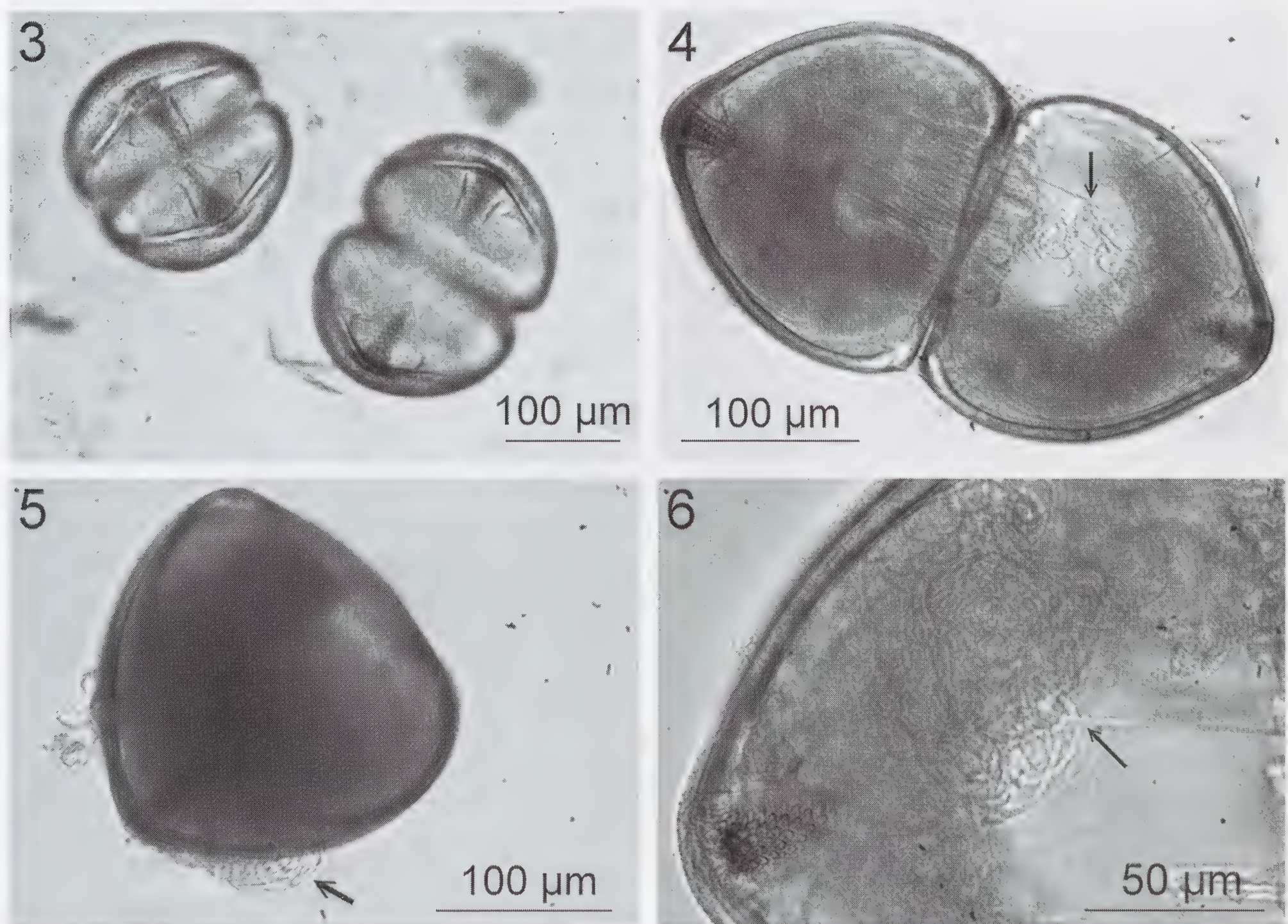
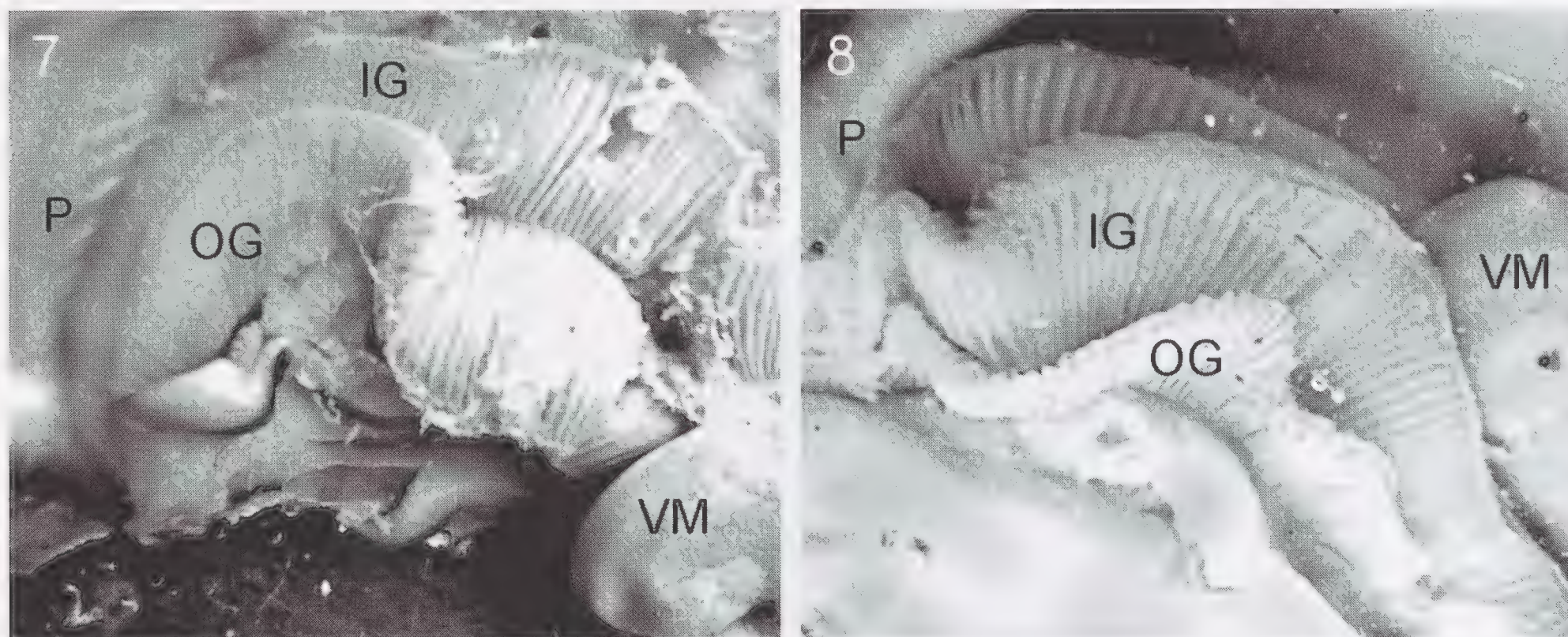


FIG. 2. Glochidial shell length and height (mean $\pm$ sd) of three *U. abyssinicus* and one *U. mancus* females from the Blue Nile (twenty measurements per female).



FIGS. 3–6. Glochidia of the genus *Unio*. FIGS. 3, 4: *U. abyssinicus*; FIGS. 5, 6: *U. mancus dembeae*. Arrows point to the larval thread.



FIGS. 7, 8. Gills of gravid *Etheria*. FIG. 7: tetrabranchous; FIG. 8: endobranchous (IG = inner gill, OG = outer gill, P = posterior end, VM = visceral mass). Note the marked swelling of the posterior endobranchous gill in Fig. 8.

Of the 25 females, 14 had been gravid in the outer gills with various developmental stages of offspring. Hardly any unfertilized eggs were observed. Three specimens contained mature glochidia. Their shape is triangular with a mean shell length of 196 μm (Fig. 2), with prominent hooks and a larval thread (Figs. 3, 4).

Unio mancus dembeae

The only specimen we found contained mature glochidia and unfertilized eggs in the outer gills. However, the percentage of glochidia was very low, namely 21% ($n = 257$ eggs analysed). Glochidia are triangular, and like *U. abyssinicus* a little bit higher than long (Fig. 2), with hooks and a larval thread (Figs. 5, 6).

Etheria

In the first study period, out of 55 specimens with a shell length between 5 and 10 cm, 28 were gravid. All females were tetrabranchous, meaning the eggs are brooded in all four gills (Fig. 7). During the second study period, 11 gravid females were tetrabranchous and 5 were endobranchous (Fig. 8). Among individuals, the stage of offspring development was somewhat different. During the first study period, only one specimen contained larvae at an early developmental stage. They were lasidia, with an undivided spherical cuticula of 60 μm diameter and two prominent ciliated anterior lobuli (Figs. 9, 10). The structures at the posterior end are apparently hooks (Fig. 11), as described for all lasidia.

During the second study period, all tetrabranchous females contained just eggs, whereas in some endobranchous females lasidia were found. Egg size was similar in both groups (Fig. 12). The lasidia of the endobranchous specimen are marked by an anterior organ of unknown function (Fig. 13).

DISCUSSION

Reproduction of *Unio*

The genus *Unio* is widely distributed in the palaearctic. The study of its taxonomy is still an ongoing task (Araujo et al., 2005; Khalloufi et al., 2011). However, within this group, a pretty clear and uniform pattern emerges with respect to the reproductive strategy: all investigated species have been found to be largely of separate sex, with an equal ratio, and the glochidia are brooded in the outer gills (Hochwald, 1988; Araujo et al., 2005). There is a critical population density below which fertilization of eggs declines (Hochwald, 1988). During the reproductive season of some months, there are several spawnings and refillings of marsupia, which are not synchronized within a population (Hochwald, 2001). The hooked glochidia are triangular, with a long larval thread (reviewed in Reis & Araujo, 2009), and their size is intermediate among glochidia (Bauer, 1994).

All these results about *Unio* are from palaearctic and northwest African species. However, they are confirmed by the data from the two *Unio* species in the Blue Nile.



FIGS. 9–11. Lasidia of a tetrabranchous *Etheria* (2009). FIG. 9: Frontal view of a larva still encapsulated; FIG. 10: Free lasidium from above (C = cuticula, L = anterior ciliated lobuli); FIG. 11: Free lasidium. The arrow points to the posterior hooks.

Unio abyssinicus is dioecious (Table 1). Only about half of the females were gravid, arguing for an extended reproductive season probably with several spawnings. Furthermore, the considerably different developmental stages of eggs indicate that reproduction is not synchronized within the population. Glochidial size is at the lowest limit thus far reported for *Unio* (Fig. 2).

The only individual of *Unio mancus dembeae* contained mature glochidia in the outer demi-branches; however, most of the eggs (79 %) had not been fertilized. With a shell length of approximately 212 µm (Fig. 2), its glochidia are smaller than the glochidia found in Iberian and Italian *U. mancus* populations (217–224 µm; reviewed in Araujo et al., 2005). It was the rarest naiad in the surveyed area. Together with the high rate of unfertilized eggs, this argues that *U. mancus* is also dioecious in the Blue Nile, as reported in Iberian populations (Araujo et al., 2005) and that the rate of fertilization declines below a critical population density, as reported for European *U. crassus* Retzius, 1788, populations (Hochwald, 1988). However, whereas in Europe it is a summer breeding species (Castagnalo, 1977; Araujo et al., 2005), in the Blue Nile reproduction occurs at least during January and February. The different breeding season, in connection with the comparatively small glochidium, might render *U. mancus dembeae* a potential candidate for a new *Unio* species if more data are available.

Reproduction of *Etheria*

A serious contradiction to published data concerns the mode of brooding of *Etheria*. Thus far the Etherioidea have been reported to be endobranchous, and this trait is considered to be a systematic characteristic of this superfamily (Graf & Cummings, 2006; Walker et al., 2006; Varjabedian, 2006; Herrmann, 1995; Artaega, 1994; Heard & Vail, 1976). But during the first study period, I found all gravid individuals of *Etheria* with eggs in all four demibranchs (tetrabranchous; Fig. 7).

During the second study period, where the mussels had been collected approximately 10 km downstream, I found 16 gravid females: 11 were tetrabranchous, 5 were clearly endobranchous (Fig. 8). Whereas egg size was similar (Fig. 12), only the endobranchous females contained ripe larvae. So far, the Etheriidae are considered to contain only one species with a large distribution area (Bogan, 2008). However, many forms have been described (Yonge, 1962), and my results indicate different reproductive modes. Therefore, I think that the genus *Etheria* comprises several species.

In 2009, out of 57 *Etheria* individuals examined, 28 were found to be gravid. This argues for an equal distribution of separate sexes, confirming the results of Ampofo-Yeboah et al. (2009) about *Etheria* in Ghana. The same condition has been found among other African

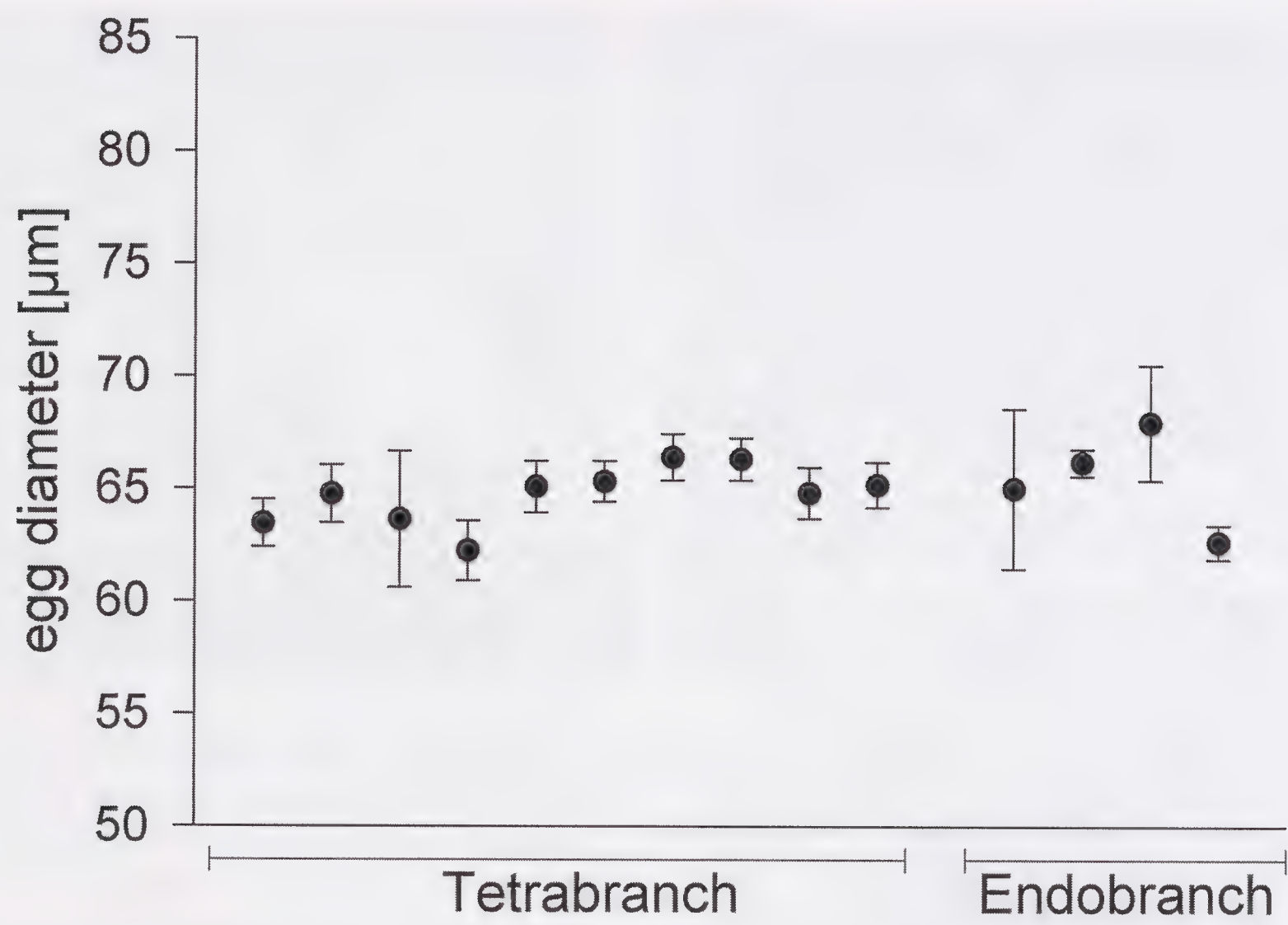


FIG. 12. Egg size (mean $\pm$ sd) of tetrabranchous and endobranchous *Etheria* collected 2010.



FIG. 13. Lasidium of an endobranchous *Etheria* (C = cuticula, L = anterior lobuli). The arrow points to an “anterior organ”, which might be related to the “ribbon like organ” of South American lasidia.

Etherioidea collected in the Nile River in Egypt, namely *Mutela dubia nilotica* Crosse, 1881, *M. bourguignati* Bourguignat, 1885, and *Chambar-dia rubens* (Savigny, 1827) (Falk, 1995; Walker et al., 2006). However, *M. rostrata* (Rang, 1835) is typically hermaphroditic. In South America, two Mycetopodidae were found to be hermaphroditic (Walker et al., 2006).

Since apparently all females were gravid during my first study period, reproduction seems to be largely synchronized, and the reproductive season in the upper Blue Nile is during low water level in January and February, confirming the results of Heard & Vail (1976) from Zaire.

Compared to the few records about the size of lasidia (Fryer, 1961; Parodiz & Bonetto, 1963; Varjabedian, 2006), the one of *Etheria* is among the smallest (60 µm, Figs. 9–11, 13).

A larval thread, as in the iridinid *M. bourguignati* was not found. However, the larvae of endobranchous females (Fig. 13) develop a structure that might be related to the anterior ribbon like organ of attachment of South American mycetopodid lasidia (Parodiz & Bonetto, 1963; Bonetto, 1997). From tetrabranchous females, I got only larvae at an early developmental stage (Figs. 9–11). Therefore, it cannot be excluded that also these larvae develop this organ later. A prominent ribbon like organ is also present at the lasidium of *Acostaea rivoli* (Deshayes, 1827), the South American “freshwater oyster” (Artaega, 1994).

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THE RADULA OF THE EXTINCT FRESHWATER SNAIL
AYLACOSTOMA STIGMATICUM (CAENOGASTROPODA: THIARIDAE)
FROM ARGENTINA AND PARAGUAY

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INTRODUCTION

Aylacostoma stigmaticum is a little known endemic freshwater snail described by Hylton Scott (1954) from specimens collected in Isla Ibicuy, an island in the High Paraná River (27°16'–27°19'S, 56°04'–56°14'W) at the border area between Argentina and Paraguay. However, at present, the Isla Ibicuy, as well as many others islands of this Paraná area (Neiff, 1986), have disappeared due to damming and the filling up of the Yacyretá Reservoir in the early 1990s (Quintana & Mercado Lackzó, 1997; Meichtry de Zaburlín et al., 2010). Because of flooding, the three endemic *Aylacostoma* species occurring in the High Paraná River [i.e., *A. guaraniticum* (Hylton Scott, 1953), *A. chloroticum* Hylton Scott, 1954, and *A. stigmaticum*], which are strictly confined to rapids, have been threatened with extinction (Quintana & Mercado Lackzó, 1997; Vogler, 2012). *Aylacostoma stigmaticum* is listed by IUCN as “Extinct in the Wild” (Mansur, 2000); but could presently be categorized as extinct, as no specimen of this species has been reported to have survived in nature or captivity. It is important to mention that an ongoing *ex situ* conservation program was developed in the 1990s. This program known, as the “*Aylacostoma* Project”, is being conducted by the National University of Misiones (UNaM, Argentina) jointly with the Argentine Museum of Natural Sciences (MACN), and is supported by the Entidad Binacional Yacyretá (EBY, Argentina-Paraguay). Currently, only *A. chloroticum* and a recently recorded new morphotype are included in this program.

The taxonomic description of *A. stigmaticum* was based solely on conchological features (according to typological approaches of most of the mid-20th century authors), especially: a conical to ovate shell, yellow horn color, low spire, last whorl somewhat convex; surface almost smooth, last whorl sculptured by low spiral

cords, a spiral band of reddish spots, and presence of irregular black spots on the surface of all specimens. The latter was stated to be the most conspicuous characteristic (Hylton Scott, 1954; Fig. 1). However, the anatomy of *A. stigmaticum* was not described and only dry shells are presently available from museum collections.

In this note, the radula of the extinct snail *A. stigmaticum* is described for the first time as a contribution to future comparative studies of *Aylacostoma*, a neotropical genus which has not been comprehensively revised yet.

MATERIALS AND METHODS

The radula was obtained from a dry syntype of *A. stigmaticum* deposited in the La Plata Museum (MLP 10965) following a modification of the method described by Holznagel (1998). The shell was rinsed with deionized water and placed in a 10 ml tube, 1.5 ml of NET buffer (10 mM Tris, 10 mM EDTA, 2% SDS) and 10 µl of proteinase K (20 mg/ml; Finnzymes #F202) were added. Solution was incubated at 37°C for seven days, adding an equal volume of proteinase K on the third day. After incubation, a gelatinous mass containing the radula was recovered from inside the shell. This tissue was incubated at 56°C with 500 µl of NET buffer and 10 µl of proteinase K for 24 hs. The cleaned radula was recovered from this incubation solution, and rinsed for four times with deionized water, mounted, and photographed using a scanning electronic microscopy (SEM). The terminology used follows Simone (2001) and Gomez et al. (2011). The radular formula is described as in Gomez et al. (2011): rachidian (number of left side cusps/median cusp/number of right side cusps); lateral teeth (inner cusps/pronounced cusp/outer cusps); marginal teeth (number of cusps on inner marginal tooth, and number of cusps on outer marginal tooth).



FIG. 1. Syntype of *A. stigmaticum* Hylton Scott, 1954 (MLP 10965).

RESULTS

The radula of *A. stigmaticum* is taenioglossate, short, forms an “S”, comprises about 75 rows of teeth (Fig. 2). Rachidian teeth pentagonal, broader than longer, with a v-shaped base; most rachidians bear seven pointed apical cusps (3/1/3), the central cusp being noticeably longer, lanceolate (Figs. 3, 4); in some rows of teeth the number of cusps varied from six to eight (formulas 3/1/2, 3/1/4; Fig. 5); rachidians with two basal cusps at each outer corner (Fig. 6). Lateral teeth long, in general with six to seven pointed cusps, the second one being larger, broad, rectangular, with asymmetrically lobate margin (1/1/4–5; Figs. 3–5); teeth of some rows with cusps showing different levels of fusion, occasionally developing bifid points. Marginal teeth similar to one another, long, spatulate, with slender, fringe-like cusps. Inner marginal teeth with about nine cusps; outer marginals with about 12 cusps.

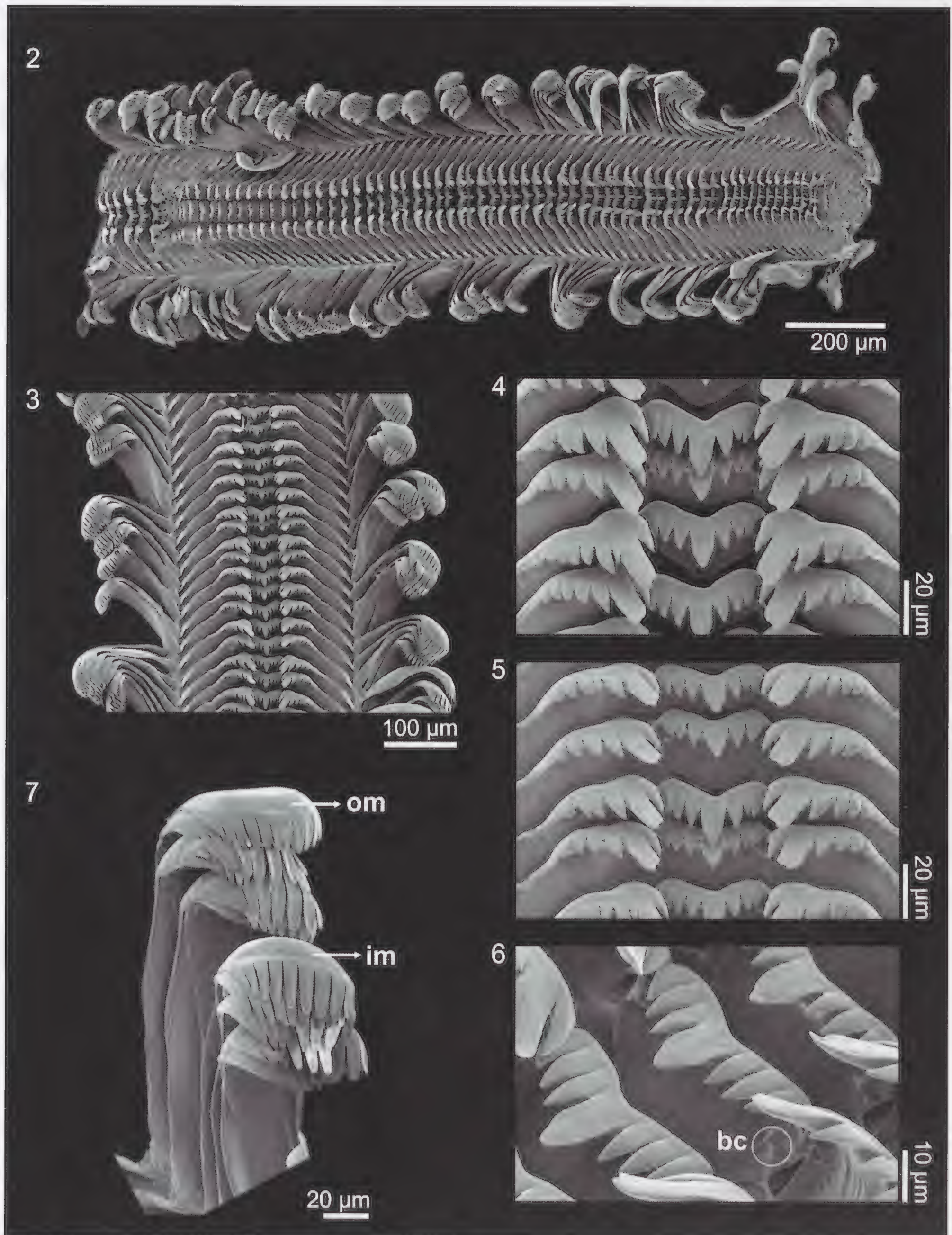
DISCUSSION

The radula of *A. stigmaticum* described here for the first time, is similar to that of other *Aylacostoma* species in having the same number

of cusps on central teeth. However, it differs from *A. guaraniticum* from Argentina-Paraguay described by Hylton Scott (1953) in having two basal cusps at each outer corner of central teeth instead of one. The central teeth of the three Brazilian species *A. exoplicata* Simone, 2001, *A. ci* Simone, 2001, and *A. tenuilabris* (Reeve, 1860) lack basal cusps altogether (Simone, 2001, 2011). The lateral teeth of *A. stigmaticum* are similar to *A. guaraniticum* and *A. ci* in having an enlarged second cusp instead of an enlarged third cusp as in *A. exoplicata* and *A. tenuilabris*. In addition, *A. stigmaticum* differs from all the mentioned species in having more denticles on both marginal teeth.

The Jamaican thiarid *Hemisinus lineolatus* (W. Wood, 1828), the only species of this genus whose anatomy has been described (Gomez et al., 2011), differs by having (1) rachidians with a central squarish cusp and a basal denticle at each outer corner, (2) lateral teeth with squarish, enlarged third cusp, and (3) marginal teeth with fewer denticles.

The radular characters described here complete the description of *A. stigmaticum* and can be used to differentiate the extinct species from other *Aylacostoma* species. This comparison suggests that the presence and number of basal cusps on rachidian teeth might be a



FIGS. 2–7. Radula of *A. stigmaticum*. FIG. 2: Radular ribbon; FIG. 3: Radular segment; FIGS. 4, 5: Detail showing central and lateral teeth; FIG. 6: Rachidian with basal cusps (bc); FIG. 7: Marginal teeth, inner marginal (im) and outer marginal (om).

useful character for differentiating species, at least among those from Argentina-Paraguay and Brazil, although this has to be confirmed by more evidence from further species.

Finally, when viewing the radula of *A. stigmaticum* under SEM, the basal cusps were not visible at first sight. Therefore, it is important to highlight that different approaches must be explored for examining the radulae of *Aylacostoma* species in order to confirm the presence or absence of these basal cusps.

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GEOMALACUS AND LETOURNEUXIA (MOLLUSCA, PULMONATA): A CYTOGENETIC ASSESSMENT

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INTRODUCTION

The terrestrial malacofauna of the Iberian Peninsula is extremely rich and shows the highest diversity of arionid slug species in Europe (and probably worldwide), with 30 to 50 species, including several endemic ones (Castillejo, 1998). However, the taxonomic status of several of these species remains unclear due to the extreme variability in body size and color and the lack of reliable diagnostic morphological traits (Backeljau & De Bruyn, 1990). The taxonomy of terrestrial slugs is based almost entirely on the morphology of their reproductive apparatus, which varies according to developmental stage and sexual maturation, often preventing the correct identification of juveniles at the species level (Backeljau & De Bruyn, 1990, and references therein; Backeljau et al., 1996). As a consequence, the taxonomic status of supraspecific arionid groupings, such as (sub) genera, is still controversial. This applies to the genera *Geomalacus* Allman, 1843, and *Letourneuxia* Bourguignat, 1866, which have undergone several taxonomic changes since their original descriptions.

Geomalacus presently comprises four species grouped into two subgenera: *G. (Arrudia) anguiformis* (Morelet, 1845), *G. (A.) oliveirae* Simroth, 1891, *G. (A.) malagensis* Wiktor & Norris, 1991, and *G. (Geomalacus) maculosus* Allman, 1843. The first three species are endemic to the Iberian Peninsula, whereas *G. maculosus* is also found in southwestern Ireland. Although *G. maculosus* is easily identified by its unique color pattern, *G. anguiformis* and *G. oliveirae* are very similar in their external morphology, showing only subtle differences in their reproductive organs (Rodriguez et al., 1993). Often, these two species have been identified solely from the geographical origin

of specimens (Castillejo & Rodriguez, 1991). Moreover, when Wiktor & Norris (1991) originally described *G. malagensis*, the set of existing features for the classification of Arionidae provided by Hesse (1926) was “considered unsatisfactory” (Wiktor & Norris, 1991), since this species could have been easily classified within any of the three genera of this family – *Arion*, *Geomalacus* or *Letourneuxia*. Therefore, Wiktor & Norris (1991) proposed additional diagnostic characters, and the new species was included in the genus *Geomalacus*.

The description of the genus *Letourneuxia* Bourguignat, 1866, was based on specimens from Algeria. This taxon is described as endemic from North Africa, and it has suffered successive changes in its taxonomic status, including being described as a subgenus of *Geomalacus* (Pollonera, 1890), a subgenus of *Arion* (Hesse, 1926), and, finally, acquiring generic status within the family Arionidae (Wiktor, 1983). The four nominal species, viz. *G. (L.) numidica* Bourguignat, 1866, *L. atlantica* Bourguignat, 1883, *L. maroccanus* Pollonera, 1916, and *G. (L.) turneri* Pollonera, 1890, together with *Arion moreleti* Hesse, 1884, have been synonymized with *L. numidica* by Wiktor (1983).

The debate as to whether *Letourneuxia* and *Geomalacus* should be kept in separate genera was fueled by the description of *G. malagensis*. However, even if *L. numidica* and *G. malagensis* are very similar in external morphology and color, they present two major differences in their reproductive organs: (1) *G. malagensis* has a large, thick epiphallus that is lacking in *L. numidica*, and (2) *L. numidica* has a voluminous atrium with a ligula inside, whereas the atrium of *G. malagensis* is slender and lacks a ligula (Wiktor & Norris, 1991). Because of the variability of these diagnostic features, and the fact

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that they mostly rely on fully mature individuals, additional evidence is needed to evaluate the degree of differentiation between *Letourneuxia* and *Geomalacus*.

Cytogenetic studies in slugs and snails have yielded important taxonomic insights (e.g., Vitturi et al., 2005; Colomba et al., 2009; Kongim et al., 2009, 2010). However, for slugs, these reports are exclusively based on the use of gonadal tissue for chromosome preparations. This constitutes a serious drawback as it excludes juvenile individuals in which the ovotestis is not yet fully developed. Juvenile terrestrial slugs are particularly difficult to identify considering that the taxonomy is based on the morphology of their highly variable reproductive apparatus. So, if other organs provide reliable cytogenetic results, then this traditional disadvantage would be overcome.

In the past, chromosome studies of slugs (Beeson, 1960) and of euthyneuran gastropods in general (Burch, 1965) suggested that karyological data might be useful to distinguish (sub) genus level taxa in limacid and arionid slugs. There seems to be a suggestive correspondence between haploid chromosome numbers and subgeneric groupings in the genus *Arion*: $n = 25$ in *Mesarion* Hesse, 1926, $n = 26$ in *Arion* Férussac, 1819, $n = 28$ in *Kobeltia* Seibert, 1873 and *Microarion* Hesse, 1926, and $n =$

29 in *Carinarion* Hesse, 1926 (Beeson, 1960). This observation was, amongst others, used to include *Microarion* in the subgenus *Kobeltia* (Backeljau & De Bruyn, 1990). However, no karyotypes are available for any *Arion*, and no cytogenetic study (chromosome number and karyotypic formula) has been conducted in *Letourneuxia* or *Geomalacus*.

Here we present karyotypes and a comparative karyological study of the four *Geomalacus* species and *L. numidica* after testing different somatic tissues (mouth and both optical and sensory tentacles) to evaluate their suitability for karyological studies. We also assess the contribution of cytogenetics to provide additional evidence to resolve the taxonomy of these slugs.

MATERIALS AND METHODS

Biological Material

Specimens of the five nominal species were collected in the Iberian Peninsula and Morocco as detailed in Table 1 and identified following Castillejo et al. (1994). Animals were kept alive at 4°C and fed with lettuce. Prior to the experiments (48 h), the slugs were kept at room temperature.

TABLE 1. Localities, number of specimens (n), diploid chromosome number and karyotypic formulae for the four *Geomalacus* and the *Letourneuxia* species used in this study (Sp = Spain; Pt = Portugal).

Genus/ species	Locality	n	Geographical coordinates	Chromosome number	Karyotypic formula
<i>G. oliveirae</i>	Gredos, Sp	1	40.3217°N, 5.0135°W	2n = 62	15m + 13sm + 3st
<i>G. oliveirae</i>	Gredos, Sp	3	40.3151°N, 5.0090°W	2n = 62	15m + 13sm + 3st
<i>G. oliveirae</i>	Pena de Francia, Sp	3	40.5144°N, 6.1567°W	2n = 62	15m + 13sm + 3st
<i>G. maculosus</i>	Chãos, Serra Estrela, Pt	1	40.5386°N, 7.3125°W	2n = 62	14m + 12sm + 5st
<i>G. maculosus</i>	Caldas Manteigas, Serra Estrela, Pt	8	40.3825°N, 7.5442°W	2n = 62	14m + 12sm + 5st
<i>G. maculosus</i>	Viana do Castelo, Minho, Pt	5	41. 7739°N, 8.6186°W	2n = 62	14m + 12sm + 5st
<i>G. anguiformis</i>	São Brás de Alportel, Algarve, Pt	4	37.2728°N, 7.8753°W	2n = 62	14m + 10sm + 7st
<i>G. malagensis</i>	Fonte Sesimbra, Setúbal, Pt	2	38.4761°N, 9.1143°W	2n = 62	10m + 12sm + 9st
<i>G. malagensis</i>	Guilhim, Algarve, Pt	15	37.1016°N, 7.9279°W	2n = 62	10m + 12sm + 9st
<i>L. numidica</i>	Tanger, Morocco	7	35.7844°N, 5.9011°W	2n = 62	10m + 12sm + 9st
<i>L. numidica</i>	Tanger, Morocco	2	35.7827°N, 5.8506°W	2n = 62	10m + 12sm + 9st

Chromosome Preparation

Whole individuals were submerged for 75 min in a 0.01% solution of colchicine at room temperature. Then ovotestis, mouth, and optical and sensory tentacles were dissected. Somatic tissues were chosen as representative structures with high mitotic rates: the mouth for the constant renewal of the radula by odontoblastic and membranoblastic cells and the tentacles for their ability to regenerate (Barker, 2001).

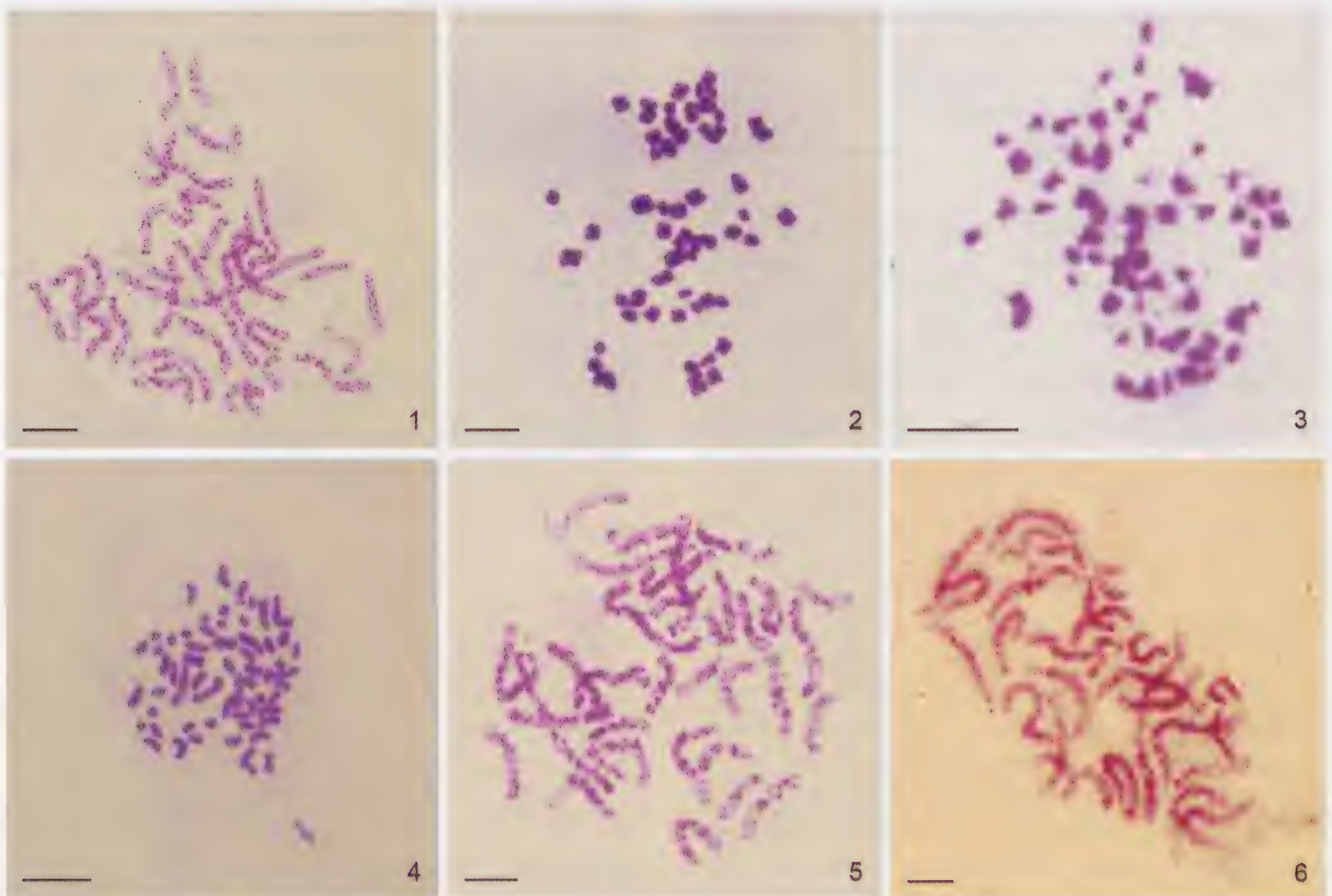
All structures were subjected to a hypotonic treatment for 45 min in 0.9% sodium citrate and fixed in a freshly prepared mixture of absolute ethanol and glacial acetic acid (3:1). Fixed pieces of ovotestis, mouth and tentacles were dissociated in 50% glacial acetic acid and distilled water. Slides were prepared following an air-drying technique (Thiriot-Quévieux & Ayraud, 1982). Slides were stained with Giemsa (4%, pH 6.8) for 10 min.

Karyotyping

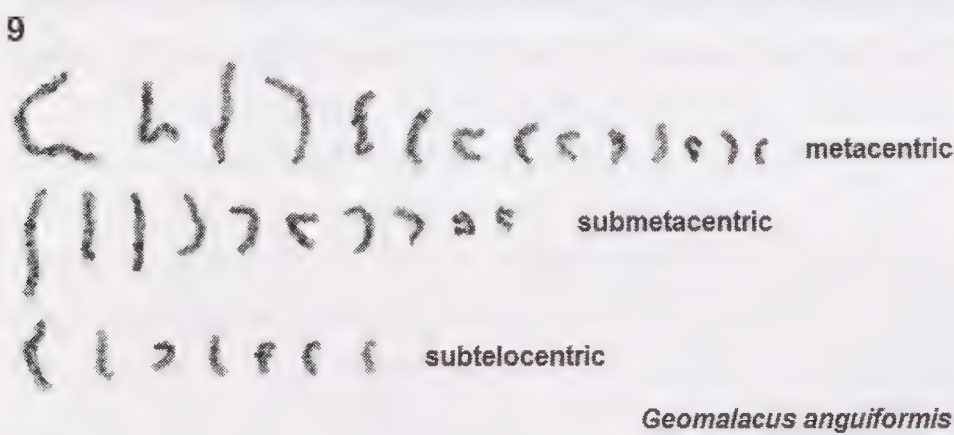
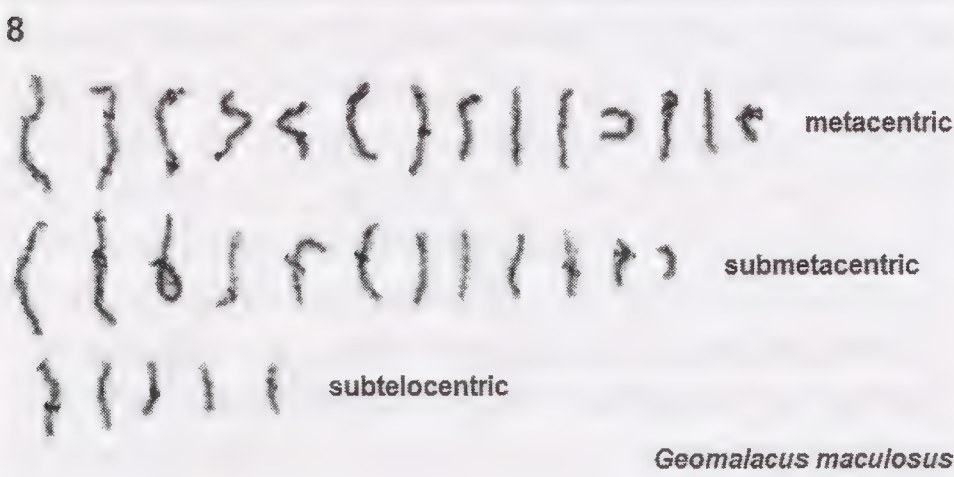
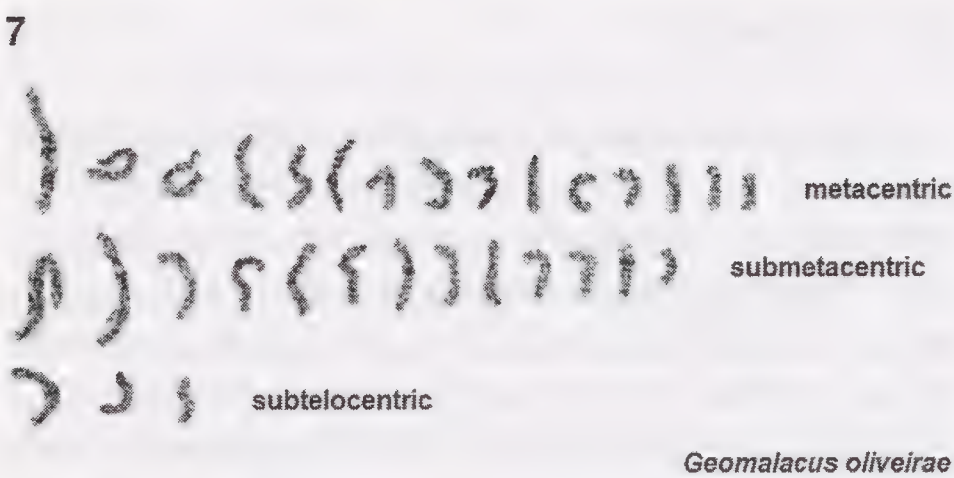
Images of Giemsa stained metaphases were acquired with a digital camera (Nikon DSFi 1) coupled to a light microscope (Nikon Eclipse 80i). Digital images were processed with Adobe Photoshop (edition CS3) using functions only affecting the whole image. Ten karyotypes per species were performed. Chromosomes were organized based on relative length and centromeric position; terminology followed Levan et al. (1964).

RESULTS

To test the suitability of different organs for producing usable chromosome images, we performed a number of trials with different individuals from the five species. Chromosomes were not obtained from preparations of

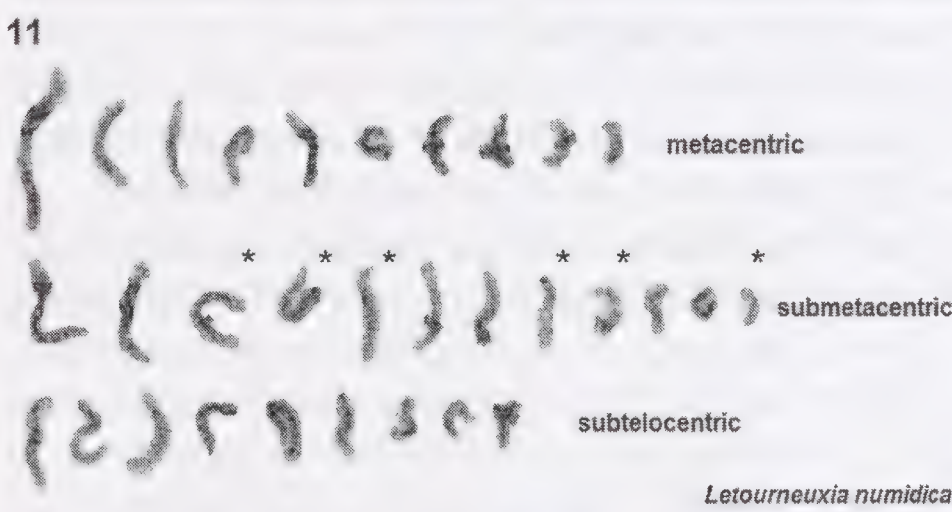
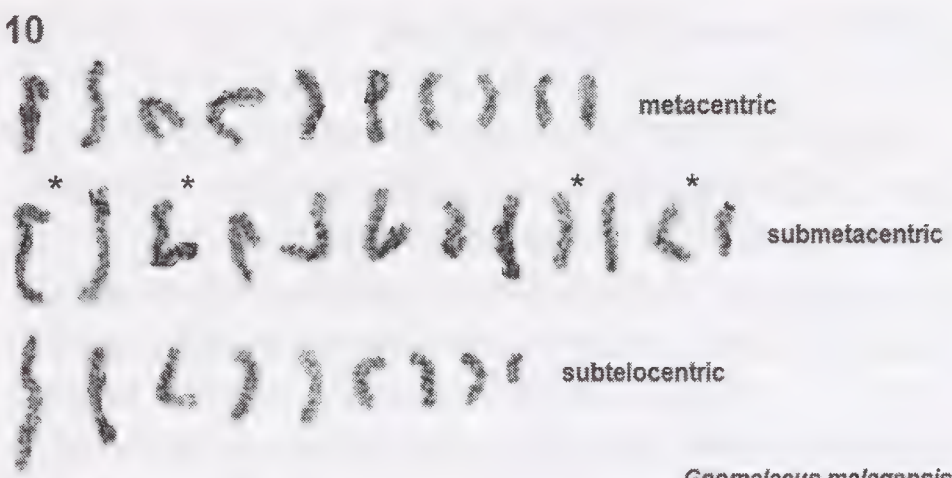


FIGS. 1–6. Giemsa stained metaphases of *Geomalacus* and *Letourneuxia*. FIG. 1: *G. oliveirae* meiotic metaphase II ($n = 31$); FIG. 2: *G. oliveirae* mitotic metaphase ($2n = 62$); FIG. 3: *G. maculosus* mitotic metaphase ($2n = 62$); FIG. 4: *G. anguiformis* mitotic metaphase ($2n = 62$); FIG. 5: *G. malagensis* meiotic metaphase II ($n = 31$); FIG. 6: *L. numidica* meiotic metaphase II ($n = 31$). Scale bar = 4 μm .



FIGS. 7–9. Giemsa stained karyotypes of *Geomalacus*. FIG. 7: *G. oliveirae* (15m + 13sm + 3st); FIG. 8: *G. maculosus* (14m + 12sm + 5st); FIG. 9: *G. anguiformis* (14m + 10sm + 7st). m - metacentric chromosomes, sm - submetacentric chromosomes and st – subtelocentric chromosomes.

ovotestis from juvenile individuals, regardless the analyzed species, but only from specimens where the ovotestis was well differentiated. However, using mouth and both optical and sensory tentacles, it was possible to obtain diploid chromosome preparations independently of the individual stage of development (Figs. 2–4). Concerning the number of chromosomes, the five species presented the same diploid chromosome number of $2n = 62$ ($n = 31$). However, karyotypic formulae are different and unique to each *Geomalacus* species, with karyotypes mainly consisting of metacentric (m) and submetacentric (sm) chromosomes; with few subtelocentric (st) and lacking telocentric chromosomes (*G. oliveirae* 15m + 13sm + 3st; *G. maculosus* 14m + 12sm + 5st, *G. anguiformis* 14m + 10sm + 7st, *G. malagensis* 10m + 12sm + 9st) (Figs. 7–11, Table 1). *Geomalacus*



FIGS. 10, 11. Giemsa stained karyotypes of *Geomalacus* and *Letourneuxia*. FIG. 10: *G. malagensis* (10m + 12sm + 9st); FIG. 11: *L. numidica* (10m + 12sm + 9st). m - metacentric chromosomes, sm - submetacentric chromosomes and st – subtelocentric chromosomes; asterisks (*) indicate submetacentric chromosomes that present a submetacentric/subtelocentric tendency.

malagensis and *L. numidica* share the same karyotypic formula, yet, in *L. numidica* six of the 12 submetacentric chromosome pairs presented a submetacentric/subtelocentric tendency, while in *G. malagensis* only four chromosome pairs show this trend (chromosomes marked with an * in Figs. 10 and 11).

DISCUSSION

The use of somatic organs for cytogenetic studies instead of ovotestis proved to be effective. Mouth and both optical and sensory tentacles yielded several mitotic metaphases and hence were successfully used to determine diploid chromosome numbers. In previous cytogenetic studies of terrestrial slugs (Beeson, 1960; Burch, 1965; Patterson, 1969; Colomba et al., 2009), only well-developed ovotestis were used for karyotyping. The new approach presented here using somatic tissues allows karyological studies to be performed regardless of the sexual developmental stage of the specimens.

The five species studied displayed an invariant chromosome number ($n = 31$) so that the observed interspecific karyotypic differences only involved structural chromosomal rearrangements without affecting chromosome number. Such patterns of chromosomal change have been previously observed in the neogastropod family Muricidae (Leitão et al., 2009). Unlike the genus *Arion*, in which chromosome numbers were useful to distinguish between subgenera (Beeson, 1960), it is clear that it is not possible to distinguish between these five species based on the chromosome number alone. The haploid chromosome number in *Geomalacus* and *Letourneuxia* is the highest observed within the Arionidae (with $n = 25$ – 29) (Beeson, 1960). Also, this chromosome number is among the highest of all terrestrial pulmonate gastropod mollusks (order Stylommatophora). Haploid chromosome numbers within this group vary between $n = 18$ and $n = 34$ (Park & Kim, 1997; Thiriot-Quiévreux, 2003; Colomba et al., 2009), with Athoracophoridae being an outlier with $n = 44$ (Patterson, 1969; Burch & Patterson, 1971).

Ancestral character state reconstruction tracing chromosome numbers in *Geomalacus* and *Letourneuxia* on a molecular phylogenetic tree, would possibly allow the inference of a chromosome number evolutionary trend for the family Arionidae. However, currently too few karyotypic data are available to conduct such analysis.

The karyotypes of the species in this study showed a prevalence of metacentric and submetacentric chromosomes, which follows the general trend in gastropod karyotypes (Thiriot-Quiévreux, 2003). Despite presenting the same chromosome number, each *Geomalacus* species displays different and diagnostic karyotypes (Figs. 1–5, Table 1). *Geomalacus oliveirae* has the most symmetric karyotype of the studied species, with the highest number of metacentric and submetacentric chromosomes, while *G. malagensis* and *L. numidica* present the more asymmetrical ones. Symmetrical karyotypes are often considered plesiomorphic, since a higher proportion of metacentric pairs may point to relative chromosomal evolutionary stability (White, 1978).

It is taxonomically relevant that *G. malagensis* is karyotypically different from its congeners, but shares the same karyotypic formula and similar chromosome morphology with *L. numidica*. The difference between *G. malagensis* and *L. numidica* resides solely in

the numbers of chromosome pairs showing a submetacentric/subtelocentric tendency, that is, respectively, 4 and 6. Even if the present chromosomal data alone is not conclusive to establish the taxonomic status of *Geomalacus* and *Letourneuxia*, this finding suggests that both genera may be closely related and perhaps should be merged in a single genus-level taxon. Similarly, Backeljau & De Bruyn (1990) used chromosome numbers, together with morphology and allozyme data, to merge the arionid subgenera *Microarion* and *Kobeltia*. Yet, whether such conclusion is also warranted for *Geomalacus* and *Letourneuxia* requires further corroboration.

In conclusion, our work showed that somatic tissues are perfectly suitable for cytogenetic studies and that the chromosome number of the genera *Geomalacus* and *Letourneuxia* is $n = 31$, which is among the highest of all Stylommatophora. The five described karyotypes constitute the first record for Arionidae, with *G. malagensis* and *L. numidica* presenting similar chromosome morphologies and karyotypic formulae. Cytogenetic studies may significantly contribute to clarify the taxonomy of these, and other, pulmonate gastropods.

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FOSSIL RECORDS OF *MARSTONIOPSIS INSUBRICA* (KÜSTER, 1853) SUGGEST ITS WIDE DISTRIBUTION IN CENTRAL EUROPE DURING THE EARLY HOLOCENE

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INTRODUCTION

The rare and endangered freshwater snail *Marstoniopsis insubrica* (Küster, 1853) (Gastropoda: Amnicolidae) today has a highly disjunct distribution in Europe (Fig. 1). It is more or less continuously distributed throughout the northern part of Europe (Glöer, 2002), where it inhabits alkaline lakes and stagnant parts of large rivers. Its status in Britain is equivocal, but some occurrences there are clearly modern introductions (Preece & Wilmot, 1979; Kerney, 1999). Isolated records are known from northern Italy (Cossignani & Cossignani, 1995) and Switzerland, but the Swiss site has now been destroyed (Turner et al., 1998). The great distance between the main and continuous range of the species and the Alpine outlier (more than 500 km away) led to the description of two distinct species: *Marstoniopsis scholtzi* (Schmidt, 1856) living in the north of Europe and *M. insubrica* (Küster, 1853), from the Alps. Recent work based on mitochondrial COI gene of these two species showed that they belong to a single species (Falniowski & Wilke, 2001), with the latter name having priority. Falniowski & Wilke (2001) suggested several scenarios of postglacial dispersal that may have resulted in this allopatric distribution. They suggested that the modern distribution did not indicate dispersal along waterways or other corridors linking northern Europe and the Alps. They also claimed that a lack of Pleistocene evidence makes it difficult to reconstruct population history and historical pathways of dispersal. A new Holocene record from Slovakia, described here, has been radiocarbon-dated to the early Holocene adding to the short list of other records from central Europe, all of which were hitherto poorly dated. These records can shed new light on the origin of its present disjunct distribution. Although rare fossil records are known in central Europe from the Eemian interglacial (Alexandrowicz & Alexandrowicz, 2010; V. Ložek pers. comm.), the critical evidence

for understanding the recent distribution are needed from the time period after the end of the last glaciation.

METHODS AND FOSSIL RECORDS

We abstracted all known published Holocene fossil records of *Marstoniopsis insubrica*, previously reported as *M. scholtzi*. The earliest Holocene record close to the modern distribution has been reported from central-east Germany by Fuhrmann (1973). Ten years later, Krolopp & Vörös (1982) published the first Holocene fossil find for Hungary, and subsequently several more records have been made throughout the western part of Hungary (Krolopp, 1986; Fűköh, 1988, 2000, 2001, 2008). Although none of these other records have been radiocarbon-dated, they appear to be early Holocene, mostly Boreal or around the Boreal/Atlantic transition (Krolopp, 1986). These ages are consistent with the new record from southern Slovakia, which is also the first for this country. The studied Parížske močiare deposits represent a full Holocene succession through a calcareous marsh, located NE of the village of Nová Vieska (47°52'25"N, 18°27'44"E) in Slovakian Danube Lowland. The age was determined using radiocarbon dating of *Carex* sp. seeds associated with the two shells of *Marstoniopsis insubrica* (Fig. 2) from 495–500 cm in the profile, the only level yielding this species. The uncalibrated date was 9760 ± 30 yrs BP (lab code UGAMS 10035), which gave a calibrated age (95.4% probability) between 11240–11165 cal. yrs BP using the IntCal09 calibration curve (Reimer et al., 2009) in the OxCal 4.1 program (Bronk Ramsey, 2009).

RESULTS AND DISCUSSION

The early Holocene records from Hungary together with the new record from southern Slo-

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FIG. 1. Distribution of *Marstoniopsis insubrica* in Europe based on available literature data: Great Britain (Kerney, 1999), Switzerland (Turner et al., 1998), Italy (Boeters, 1973; Giusti & Pezzoli, 1980; Dalfreddo & Maiolini, 2003), northern and central Europe (Falniowski, 1987; Fűköh, 1988, 2000; Glöer, 2002; Glöer & Meier-Brook, 2003; Krolopp, 1986). Distribution of other three species of the genus *Marstoniopsis* in Europe is shown: MA, *M. armoricana* (Paladilhe, 1869) known from western France (Pasco, 2005; Gargominy et al., 2011); MC, *M. croatica* Schütt, 1974, recorded at few sites in Slovenia (Schütt, 1974); and MV, *M. vrbasi* Bole & Velkovrh, 1987, known only from the type locality in Bosnia and Herzegovina (Bole & Velkovrh, 1987), which has been probably destroyed.

vakia fill a distribution gap between northern and southern present populations (Fig. 1). Because of the presence of continental ice sheet that covered much of northwestern Europe during the last cold stage, it is likely that the modern distribution of *M. insubrica* in most of northern Europe is of postglacial origin. These fossil records can therefore suggest a possible dispersal pathway of postglacial spreading from southern refugia to the north, in a southeasterly direction along the Alps, though the location of its glacial refugia are unknown. Although they cannot identify the location of refugia, they enable us to exclude some speculations about the origin of northern populations and also explain the surprisingly low genetic differentiation between northern and southern populations reported by Falniowski & Wilke (2001). Their hypothesis of passive long-distance dispersal by birds does not seem to be parsimonious. During the early Holocene, possibly up to the beginning of the Atlantic period (Krolopp, 1986), the species may have continuously occupied a large area from Italy into northeastern Europe. The age of these

records, close to the end of the last glaciation, might further support the hypothesis of periglacial survival in the Pleistocene periglacial zone of central Europe (Falniowski & Wilke, 2001). Interestingly, the southernmost fossil record from Hungary is located only 150 km from an isolated population of *Marstoniopsis* in Slovenia, originally described as a distinct species, *M. croatica* Schütt, 1974. This raises a question on the status of that species, a resolution that must await further molecular data. In this respect, another endemic species, *M. vrbasi* Bole & Velkovrh, 1987, described from a single site in Bosnia and Herzegovina (Bole & Velkovrh, 1987) also requires taxonomic reconsideration. The same is true for the fourth member of the genus, *M. armoricana* (Paladilhe, 1869), known from western France (Pasco, 2005; Gargominy et al., 2011).

In conclusion, the records of *Marstoniopsis insubrica* from the early Holocene suggest an alternative model of postglacial dispersal. It is possible, that this cold adapted species (data in Falniowski & Wilke, 2001) survived the last glacial maximum in the periglacial zone of temperate Europe and also possibly south of the Alps. After the deglaciation it might have spread northwards, but left several isolated populations in southern interglacial refugia behind (Stewart et al., 2010). Similar postglacial scenarios have been suggested for some cold tolerant *Vertigo* species that display remarkably similar distribution patterns in Europe (Kerney et al., 1983; Schenková & Horsák, 2013). As these four recently isolated southern populations of *Marstoniopsis* were originally described as distinct species, further molecular research is needed to resolve the question of their refugial character. We should test a hypothesis that these geographically limited species might have originated from much wider distribution of *M. insubrica* in central and southern Europe during the Late Glacial and early Holocene.

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FIG. 2. Shell of *Marstoniopsis insubrica* (Küster, 1853) from the Parížske močiare Holocene succession (SE Slovakia); the material is deposited in M. Horsák's personal collection (Brno). Shell height/width: 1.80/1.25 mm. Photo by M. Horsák.

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REGRESSIONS FOR ESTIMATING GASTROPOD BIOMASS WITH MULTIPLE SHELL METRICS

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INTRODUCTION

Snails are among the most important primary consumers in freshwater ecosystems. They eat large amounts of periphyton, facilitate nutrient regeneration for the resources they consume, and in ecosystems with greater than two trophic levels, snails are a vital conduit for energy flow to upper trophic levels by serving as prey to numerous invertebrate and vertebrate predators (Dillon, 2000). Estimates of individual biomass (mass/individual) enable calculations of standing stock (mass/area) and production (mass/area/time). These measures are superior to density (no./area) for assessing the influence of animals on communities and ecosystems because they incorporate size-structured aspects of energy flow (Regester et al., 2006; Rosas-Luis et al., 2008).

Length-mass regressions enable quick estimation of individual biomass to assist estimating standing stock and production. Regressions provide mass estimates without sacrificing animals, which may not be feasible for protected taxa or desirable because repeatedly sampling the same population can cause bias (Cahill et al., 2002). Obtaining biomass data from preserved specimens is facilitated by regressions because length is a relatively stable measure compared to mass (Wetzel et al., 2005). However, historically most studies of freshwater snails report density without measures of biomass (Garner & Haggerty, 2010; Ruehl & Trexler, 2011). Among those studies that report biomass data, few report regressions that include more than one measure of length (e.g., shell length or width) or mass (e.g., wet mass or ash-free dry mass, a measure of organic content) and rarely combine multiple measures of length to improve mass estimates (e.g., Eckblad, 1971; Boerger, 1975; Collins, 1992; Benke et al., 1999; González et al., 2002; Deichmann et al., 2008). Combining multiple length measures should improve predictive

power and provide more accurate biomass estimates for snails because they exhibit a diversity of shapes. Comparisons among possible regression models may reveal that different metrics or combinations of metrics are better predictors of a particular mass measure for a given group (e.g., AFDM vs. wet mass).

In this study, we consider three common, structurally diverse freshwater snail genera *Planorbella*, *Pomacea* and *Haitia* to examine the utility of using multiple length measures to predict multiple soft-tissue mass measures. *Planorbella* (Planorbidae) are pulmonate snails with planispiral shells that grow sinistrally (left handed). *Haitia* (Physidae) are small pulmonates with a sinistrally growing conspiral shell. *Pomacea* (Ampulariidae) are large caenogastropods that have globose, conspiral shells that are generally dextral (right handed) with an operculum. Additionally, we report the first length-to-mass regression for *Pomacea flagellata* Say, 1827.

METHODS

We collected *P. duryi* Wetherby, 1879 (7.67–23.46 mm shell length, $n = 89$) and *H. cubensis* Pfeiffer, 1839 (4.58–9.25 mm shell length, $n = 30$) from populations maintained at the Daniel Beard Research Center in Everglades National Park. These populations originated from collections made throughout the Everglades and were supplemented annually with wild-caught individuals. *Pomacea paludosa* Say, 1829 (10.33–44.43 mm, $n = 19$) were obtained from live-caught animals and their offspring. *Pomacea paludosa* sample size was comparatively small, but we believe it is sufficient for our purpose because preliminary regressions indicated that model fit was very good. *Pomacea flagellata* (6.8–51 mm, $n = 49$) were collected from Mexico (Sian Kaan, Quitana Roo, 19.20°N, 87.89°W) and Belize

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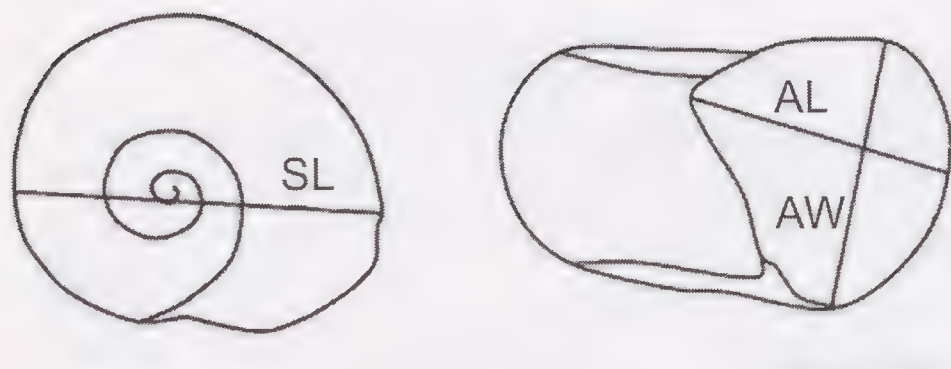


FIG. 1. Graphical description of how aperture width and length, and shell length were measured *P. duryi*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, aperture width is the length across the aperture at its widest point using calipers to 0.01 mm. Scale bar = 6 mm.

(Crab Catcher lagoon, Orange Walk 17.65°N, 88.62°W). Facilities were not available to get dry and ash mass of *P. flagellata*. All snails were sacrificed by freezing for 24 h (Nielsen & Gosselin, 2011).

Shell measurements were made to the nearest 0.01 mm with calipers and weighed to the nearest 0.0001 g. Shell length and aperture width and length were measured on *Planorbella* (Fig. 1) and *Haitia* (Fig. 2). We measured shell length, aperture width and length as well as operculum width and length on *P. paludosa* (Fig. 3). After measurements, the soft tissue was separated from the shell with forceps. Soft tissue and shell were weighed and then dried at 60°C to a constant mass. Dried soft tissue, shell, and opercula were weighed and then combusted at 500°C for four hours in a muffle

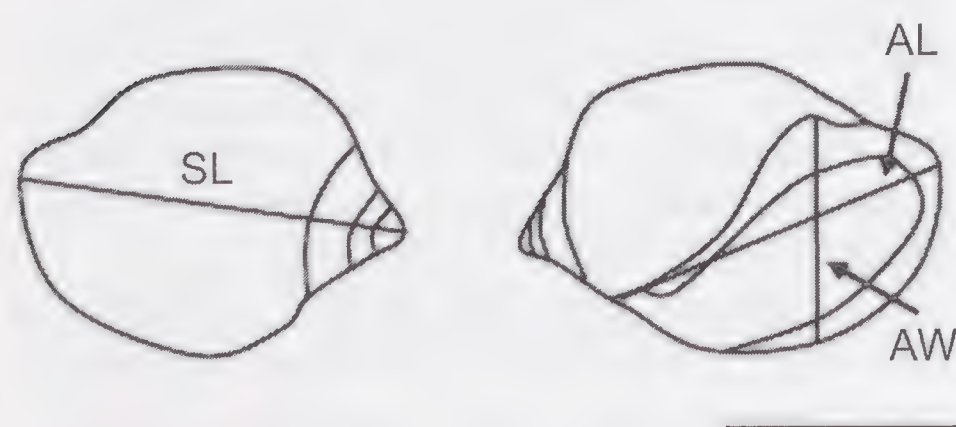


FIG. 2. Graphical description of how aperture width and length, and shell length were measured for *H. cubensis*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, and aperture width is the length across the aperture at its widest point using calipers to 0.01 mm. Scale bar = 3 mm.

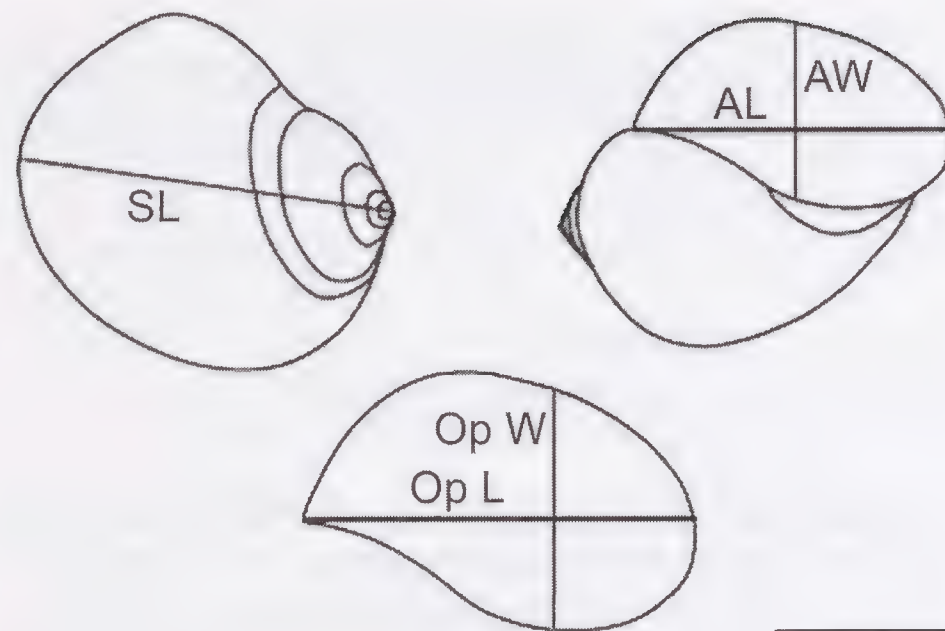


FIG. 3. Graphical description of how aperture and operculum width, length, and shell length were measured for *P. paludosa* and *P. flagellata*. Shell length is the lateral distance from anterior to posterior, aperture length is the distance across the aperture from anterior to posterior, aperture width is the length across the aperture at its widest point and opercula length and width that correspond with aperture length and width measurements using calipers to 0.01 mm. Scale bar = 10 mm.

furnace and reweighed. We subtracted the ashed mass from the dried mass to calculate AFDM.

We quantified the capacity for multiple length measures to predict mass compared to single measures using a model selection procedure with regression. Regressions were used to determine relationships between mass (wet, dry, and AFDM) and shell size (length, width, operculum length, operculum width). We tested all metric combinations to find the best model for each measure of mass for each species. Models were compared using the information-theoretic approach proposed by Burnham & Anderson (2002). Each model was ranked relative to the others using second order Akaike's Information Criterion for small sample sizes (AIC_c) where the lowest value indicated the best fit. The AIC_c calculation adds another parameter to the AIC formula that further penalizes model fit for including more independent variables. Models were considered equal if the difference between the calculated AIC_c value and the lowest AIC_c value for each dataset was ≤ 2 (Compton et al., 2002). Model weights facilitated better resolution of models with AIC_c values ≤ 2 . Data were natural-log ($\ln$) transformed because a plot of mass by length suggested a power relationship.

RESULTS AND DISCUSSION

The information-theoretic approach revealed that in many cases, multiple measures of shell morphology predicted tissue mass better than single shell measurements. Model weights, the relative probability of model accuracy, indicated that inclusion of a length and width parameter provided more robust models (Table 1). Even when the $\Delta AICc$ values for models with only one parameter were low, the model weight was correspondingly low meaning that it was unlikely that single parameter models provided the best fit. Inclusion of extra parameters also increased the R^2 value (Table 2). For the two pulmonate species, the best combination of

measurements changed depending on the mass measurement, but always included shell length. Wet mass was predicted best by shell length and aperture length or width for *P. duryi* (Table 1). Similarly, a combination of shell length and aperture width, or these and aperture length, predicted wet mass best in *H. cubensis*. However, models that included only shell length and aperture width predicted dry mass best for *P. duryi*, while shell length with aperture length best predicted dry mass in *H. cubensis*. Finally, a combination of shell length and aperture width best predicted AFDM for both *P. duryi* and *H. cubensis*. Therefore, regardless of mass measurement, we recommend gathering data on shell length and ap-

TABLE 1. Lowest AIC values out of all possible model variations including shell length (SL), aperture width (AW), aperture length (AL), operculum length (OpL) and operculum width (OpW) for each species and mass measure. Multiple models are included if $\Delta AIC \leq 2.5$. Model weight is the relative likelihood a given model provides the best fit.

Species	Mass Measure	Parameters	$\Delta AICc$	Model Weight
<i>P. duryi</i>	Wet	SL, AW, AL	0	0.65
	Wet	SL, AL	2.12	0.22
	Dry	SL, AW	0	0.61
	Dry	SL, AW, AL	2.21	0.2
	AFDM	SL, AW	0	0.63
	AFDM	SL, AW, AL	2.28	0.2
<i>H. cubensis</i>	Wet	SL, AW	0	0.71
	Wet	SL, AW, AL	1.91	0.27
	Dry	SL, AL	0	0.68
	Dry	SL, AL, AW	2.4	0.2
	AFDM	SL, AW	0	0.3
	AFDM	SL	0.16	0.28
	AFDM	SL, AL	0.35	0.25
	AFDM	SL, AW, AL	1.62	0.13
<i>P. paludosa</i>	Wet	OpL	0	0.36
	Wet	AL, OpL	2.4	0.11
	Dry	AW, OpL	0	0.26
	Dry	OpL	1.31	0.13
	Dry	AW, AL	1.39	0.13
	AFDM	AW, OpL	0	0.35
	AFDM	OpL	2.62	0.09

TABLE 2. Top two regression equations as selected by AIC and the equation for shell length (SL) to provide a comparison with the exception of *H. cubensis* AFDM where SL was one of the top two regression equations. Regressions are of transformed SL, aperture width (AW), and length (AL) and operculum width (OpW) and length (OpL) to ln transformed wet mass, dry mass and AFDM for *P. duryi* (n = 89), *P. paludosa* (n = 19) and *H. cubensis* (n = 30).

Mass	<i>P. duryi</i>	<i>P. paludosa</i>	<i>H. cubensis</i>
Wet	1.16 SL + 0.66 AW + 0.77 AL - 7.07 R ² = 0.87	3.09 OpL - 7.97 R ² = 0.98	2.14 SL + 0.87 AW - 8.96 R ² = 0.73
	1.31 SL + 1.1 AL - 6.94 R ² = 0.86	0.88 AL + 2.23 OpL - 7.99 R ² = 0.98	2.37 SL + 0.96 AW - 0.36 AL - 8.96 R ² = 0.73
	2.45 SL - 7.96 R ² = 0.84	2.85 SL - 8.33 R ² = 0.97	2.83 SL - 9.34 R ² = 0.67
Dry	1.72 SL + 0.8 AW - 9.49 R ² = 0.81	-1.62 AW + 4.51 OpL - 9.43 R ² = 0.92	1.91 SL + 0.36 AL - 9.31 R ² = 0.68
	1.65 SL + 0.75 AW + 0.1 AL - 9.41 R ² = 0.80	2.89 OpL - 9.01 R ² = 0.91	1.89 SL + 0.1 AW - 1.44 AL - 12.06 R ² = 0.67
	2.28 SL - 9.66 R ² = 0.80	2.65 SL - 9.34 R ² = 0.89	3.13 SL - 12.18 R ² = 0.64
AFDM	1.53 SL + 1.34 AW - 10.27 R ² = 0.66	-1.33 AW + 4.32 OpL - 9.92 R ² = 0.96	2.01 SL + 0.54 AW - 10.81 R ² = 0.5
	1.54 SL + 1.35 AW - 0.02 AL - 10.29 R ² = 0.66	3.0 OpL - 9.62 R ² = 0.95	2.44 SL - 11.05 R ² = 0.49
	2.48 SL - 10.57 R ² = 0.64	2.80 SL -10.08 R ² = 0.93	

erture width for species that are shaped like *P. duryi* or *H. cubensis*, and adding the aperture length measure to increase accuracy. Adding this extra measure requires little effort but provides three-dimensional information on shell shape and improves biomass estimates.

Presence of predators and variable nutrient concentrations often alter shape and life history traits that may influence length-mass regressions. Predators induce different shell shapes, shell thickness, and life history traits in both physid and planorbid snails (DeWitt et al., 2000; Hoverman et al., 2005; Auld & Relyea, 2008; Ruehl & Trexler, 2013). Differential allocation of resources represents a tradeoff with reproductive effort and is situation dependent with a size limited predator: in times of low resources, individuals will mature early and risk predation to reproduce quickly, while in times of abundant resources individuals will attempt to grow to a size refuge before reproducing (Chase, 1999; Crowl & Covich, 1990). Snails that differentially allocate resources or induce shape variation may affect the fit of length-weight regressions

by adding variability. For example, tissue mass within a globose shell may weigh the same as tissue from a longer shell but not be consistent with the relationship between the two established by the regression. A similar case might exist if more resources were allocated to shell thickness than tissue growth. Caution should be exercised when applying these regressions to samples taken in areas with only one predator type or during extremes in resource availability (e.g., eutrophication, drought). Despite this limitation, these general regressions are applicable for studies calculating energy budgets for ecosystems or energy flow in food webs.

Comparing regressions for *P. paludosa* revealed that operculum length alone or combined with aperture width or length yielded the lowest AICc for all mass measures. While all three models were equivalent according to the AICc value (Table 1), model weights indicated OpL had the best fit for wet mass, OpL and AW gave the best fit for dry mass and AFDM. Therefore, for two of the metrics multiple measures were the best predictors.

Some *Pomacea* species have shown sexual dimorphism (Cazzaniga, 1990) that may have affected the accuracy of our regressions. However, the reported R^2 values were higher than those found in the literature: Sykes (1987) and Cattau et al. (2010) documented a R^2 of 0.75 and Bourne & Berlin (1982) found a maximum R^2 of 0.56. The regressions from this study may bias results if estimating mass for one gender, but this scenario is not likely for field collections because even though brood sex ratios are highly variable in *Pomacea* the population sex ratio is not (Yusa & Suzuki, 2002).

Youens & Burks (2008) found similar results to our *P. paludosa* regressions when comparing wet mass to shell height and shell length (they referred to our operculum length as operculum width). They agreed with Guedes et al. (1981) that operculum length is analogous to the base of an elaborate shell and therefore represents the most reliable measure of wet mass. Another possible explanation is that because the operculum grows from the tissue, it is more likely to be indicative of body mass. Use of operculum length for mass estimation has ancillary benefits. For example, intact opercula are easily retrieved from gut contents of molluscivores; a regression that predicts mass from operculum length provides an estimate of energy transfer through the food web and offers potential insight into size-selective predation by different molluscivores (Hulsey et al., 2006). Additionally, opercula are occasionally found in the fossil record and could provide estimates of standing stock for snails in an ecosystem from the past (Martín & Francesco, 2006). Aperture and operculum size are undoubtedly related and possibly redundant as factors, however our results show the value of including these parameters in our analysis. Models of best fit for all three mass measures include both operculum and aperture measurements. In addition, aperture and operculum length are the only parameters in one of the wet mass models. Because the AIC method penalizes additional parameters, these models would not have had low AIC scores unless both were necessary. Therefore, we felt the inclusion of both aperture and operculum measurements was important.

Only shell length and wet mass measures were available for *P. flagellata* ($y = 2.593x - 7.923$, $R^2 = 0.83$). We include this regression because it is the first reported length-mass relationship for this species. Though we do not have the same depth of information for this species as the others, the information we have reported will aid future studies on this species and the ecosystems where it lives.

The error structure in a non-linear model is assumed to be normal and additive while error structure in a log-transformed linear model is assumed to be lognormally distributed and multiplicative (Xiao et al., 2011). While there is some debate as to which error structure is most realistic for biological studies, use of log-transformed, multiplicative error structures have been found to be more reliable (Kerkhoff & Enquist, 2009; Xiao et al., 2011). Therefore we are confident in our use of log-transformed data.

Few gastropod studies report length-mass regressions for wet mass, dry mass and AFDM together (but see Collins, 1992), but studies that use biomass report data using each of the three measures. We report all three mass measures to facilitate application of the technique and because each measure potentially conveys different information. Wet mass provides an estimate of the live mass of the animal and is probably most useful when comparing among closely related taxa that have similar shapes. Dry mass includes both organic and inorganic matter in estimates and could be reported instead of wet mass when comparing among taxa that differ greatly in the water content of their tissue. Ash-free dry mass reveals the organic content of tissue, which is an indicator of the differential allocation of resources between hard and soft tissues within an individual or among taxa. Therefore, depending on the scope and level of taxonomic comparison, researchers might report different forms of mass measurements, but reporting all three regressions will enable the conversion of one to the other for meta-analytic purposes. Because snails exhibit a wide diversity of form, one single measurement is unlikely to be adequate for all taxa. To address this challenge, we recommend reporting multiple length measures that will improve comparisons among studies by increasing consistency and accuracy. Furthermore, we suggest research to examine the accuracy of these regressions to different species within genera to aid in determining the extent of their applicability. Finally, using multiple length measures simultaneously to predict biomass offers more accurate and precise estimates of snail mass to better predict the influence of snails on communities and ecosystems. We propose this study as a first step towards encouraging the use of multiple measures of shell length for predicting biomass that will facilitate estimates of standing stock and production in ecosystems for greater understanding of energy flow and ecosystem function.

ACKNOWLEDGMENTS

We thank Luis Zambrano for access to Sian Kaan biopreserve and Joel Trexler for providing resources for the project. CBR received support from the Everglades Foundation. This project was made possible by financial support from Cooperative Agreement H5000060104, Task No. J5297070024, between the U.S. National Park Service and Florida International University, and was developed in collaboration with the Florida Coastal Everglades Long-Term Ecological Research program under National Science Foundation Grants No. DBI-0620409 and DEB-9910514.

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DIFFERENCES IN SIZE AND DEPTH FOR TWO MORPHS OF
DIAULULA SANDIEGENSIS (COOPER, 1863)

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Diaulula sandiegensis (Cooper, 1863) has long been known to vary in dorsal coloration, with a ground color ranging from cream to dark brown, overlain by either rings or irregular blotches (Behrens, 1991; Smith & Carlton, 1975). Some morphs previously considered to be separate species (*Diaulula* sp. 1, *Doris* s. l.) have recently been combined into *D. sandiegensis* (Behrens & Valdés, 2001; Behrens, 2004). However, other morphs may actually be separate species (Dayrat, 2010). I investigated whether morphs common in British Columbia showed consistent differences in size or depth of occurrence.

There are at least two common forms, though each shows variation in characters, and some individuals are ambiguous in their markings (Table 1, Fig. 1). The “ring” form has few brown rings symmetrically distributed and offset from the midline, typically on a light background (Fig. 1A). These rings may be complete or broken and relatively light or dark. The “spotted” form has multiple, irregular, dark patches similar to leopard spots in that they are surrounded by a ring of different (in this case, lighter) value, and they often grade into this color towards the center. These spots are often asymmetrically

distributed on a background that is darker than both the outer ring and the outer mantle edge (Fig. 1B). The spotted pattern seems more frequent in the northern parts of its range, for example, in Washington and British Columbia (MacFarland, 1966; Morris et al., 1980; McDonald, 1983; Dayrat, 2010) and may be reflected in the common names given in guidebooks: those from California often list the species’ common name as “ring-spotted doris” (Morris et al., 1980; Ricketts et al., 1985), whereas those from the northern part of the range often use “leopard doris” (Harbo, 2011; Lamb & Hanby, 2005).

Diaulula sandiegensis were collected for other studies from February 1998 through August 2000 (Penney, 2008, 2013) by hand from the intertidal zone or using SCUBA, in Barkley Sound near Bamfield, British Columbia, Canada. Collection was haphazard with respect to size or dorsal pattern, but no ambiguous individuals were encountered. Three field sites contributed the most animals: Scott’s Bay (48°50’06”N, 125°08’48”W), Dixon Reef (48°51’24”N, 125°07’06”W) and the west side of Seppings Island (48°50’30”N, 125°12’30”W). Nudibranchs were kept in flowing, natural seawater at Bamfield Marine Sciences Centre

TABLE 1. Characters associated with *Diaulula sandeigensis* morphs. Characters listed are for typical forms; individuals may vary in the consistency of characters. “Halo” means an area lighter than the ground color of the dorsum.

Character	Ring	Spotted
Form of marking	Ring, open in middle	Spot, closed or gradient to ground color in middle
Number of markings	4–10	> 10
Size variation among markings	Little	Great, large spots > 5x diameter of small
Distribution of markings	Symmetrical	Asymmetrical
Markings on midline	No	Yes
Halo around markings	No	Yes
Central dorsum darker than edge	No	Yes

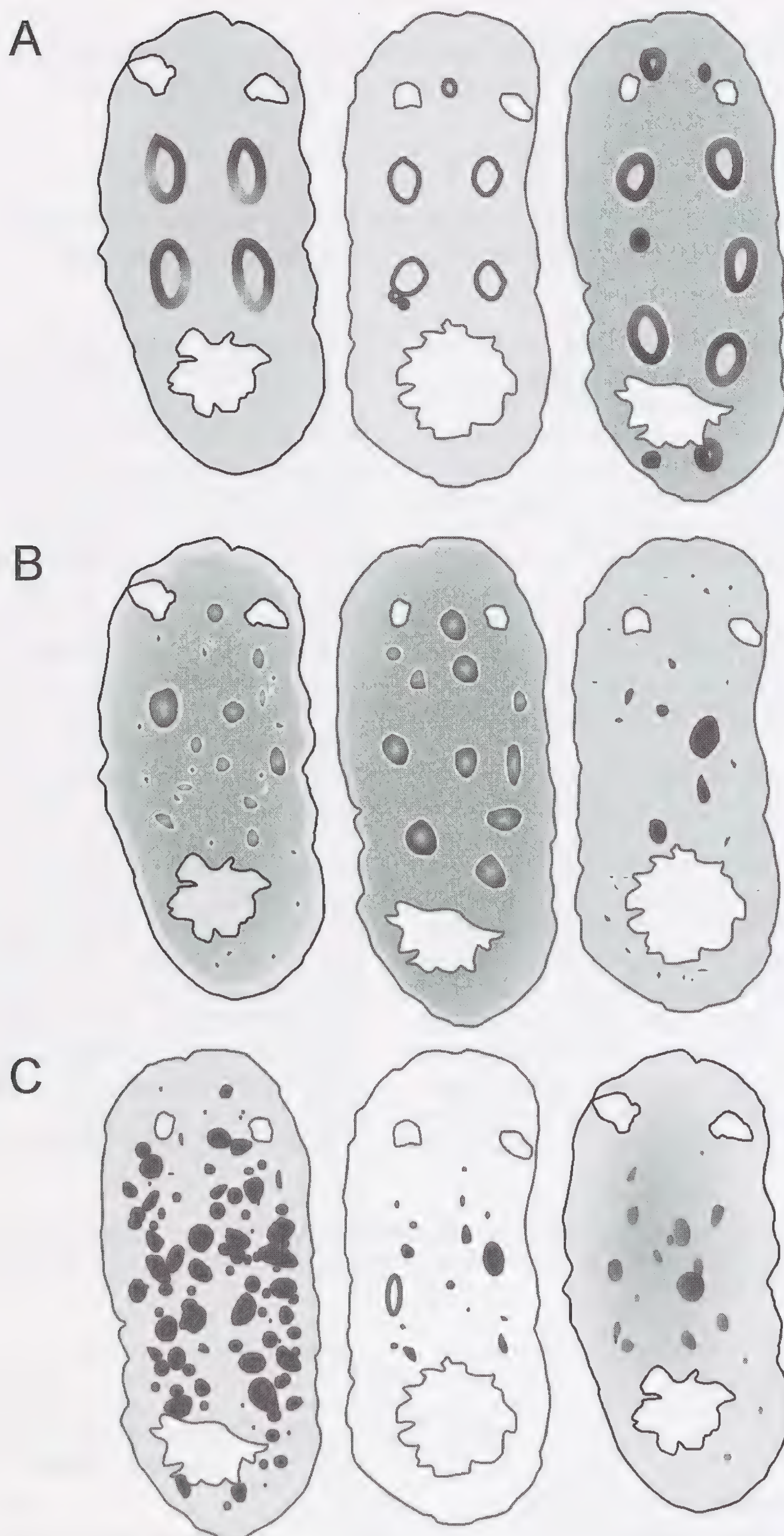


FIG. 1. Diagrams illustrating pattern differences between and variation within morphs of *Diaulula sandiegensis*. A: Ring form; B: Spotted form; C: Ambiguous: not clearly ring or spotted. Rhinophores and gill are colored white to distinguish from mantle patterns. See Table 1 for characters.

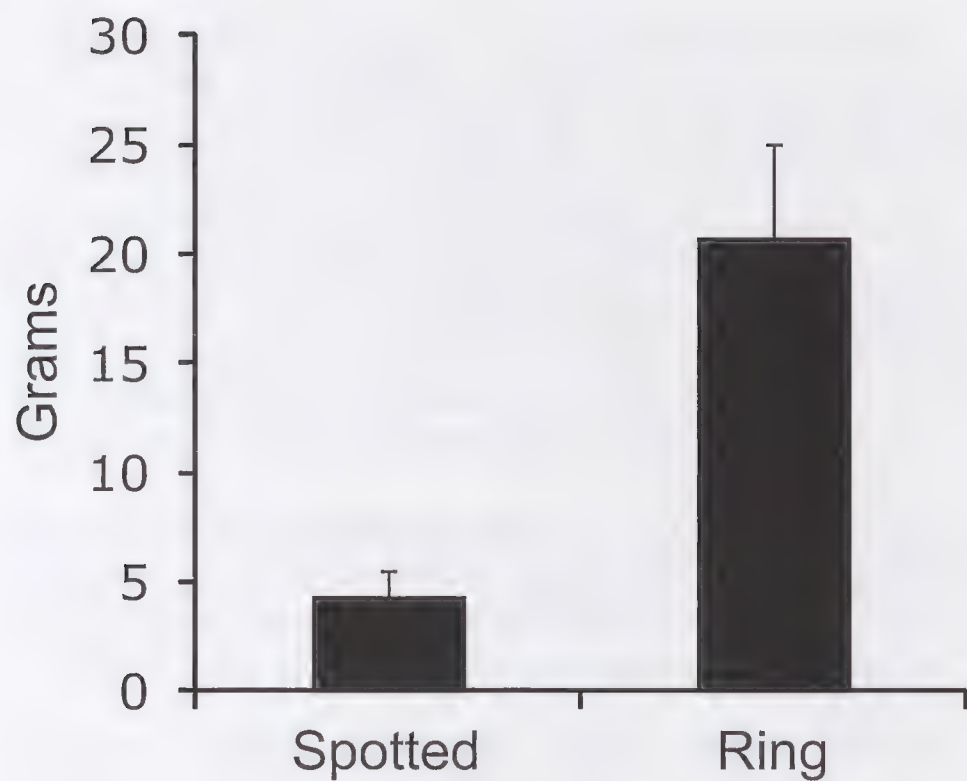


FIG. 2. Size differences between morphs of *Diaulula sandiegensis* in Barkley Sound, British Columbia.

until weighed, within three days of collection. For measurements, all animals were brought from sea table to the laboratory in their resident container. Individuals were scored as “ring” or “spotted” as defined above, damp dried on a paper towel then immediately transferred to a tared weigh boat on a calibrated digital balance.

The two morphs differed in size: spotted forms averaged 4.4 g compared to 20.8 g for ring forms (T-test, $n = 37$, $p < 0.0001$; Fig. 2). Restricting this analysis to just Scott’s Bay – the only field site where both intertidal and subtidal animals were collected – gave an equivalent result (2.4 vs. 30.2 g, T-test, $n = 22$, $p < 0.0001$). There is

some overlap in the size range for the morphs: the maximum mass found in spotted forms was 8.5 g, and two specimens of the ring form were below this weight (5.1 g, 8.1 g). Ricketts described specimens from Sitka similar to the spotted form (“darker in color, gray brown”) but larger than Monterey specimens like the ring form (Ricketts et al., 1985: 66), so size differences may depend on site and food available.

The two morphs also differed at the depth at which they were found, with spotted forms predominantly intertidal and ring forms exclusively subtidal (Fisher’s Exact Test, $n = 37$, $p < 0.0001$; Fig. 3). Again, restricting the analysis to Scott’s Bay specimens did not change the pattern (Fisher’s Exact Test, $n = 22$, $p = 0.001$). Spotted individuals were sometimes found subtidally, but no ring forms were found in the intertidal zone in this study or in over six years of observation (1997–2002). Previous studies have concluded that *D. sandiegensis* from bays are darker colored than those from open coastal waters (McDonald, 1983), and I found more of the spotted forms at Scott’s Bay than the more open waters of the Dixon Reef or Seppings Island sites. I have also found the spotted forms in protected waters at Friday Harbor, Washington. Morris et. al. (1980) noted that *D. sandiegensis* is primarily subtidal in the south and intertidal in the north of its range; instead, a spotted, intertidal morph might be primarily present in the north. Diets of the ring forms have been characterized (Penney, 2013), but similar data are not available for spotted

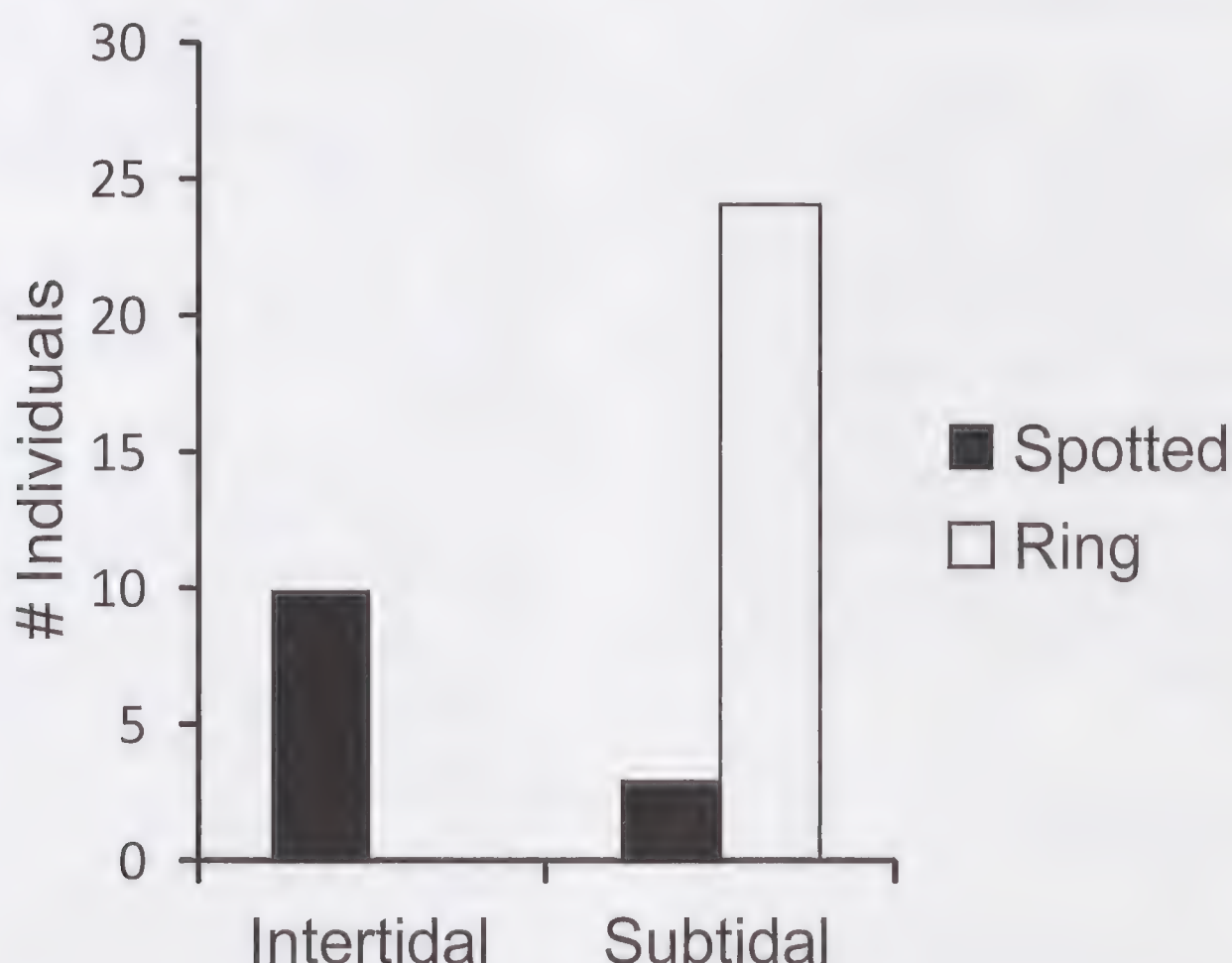


FIG. 3. Association of morphs with depth at sites in Barkley Sound, British Columbia.

forms. Intriguingly, *D. sandiegensis* chemistry also varies from north to south (Kubaneck & Andersen, 1999), although the investigators did not record dorsal pattern.

Behrens & Valdés (2001) found there were no internal anatomical differences among the *D. sandiegensis* specimens they analyzed, but the spotted form likely was not included in their analysis. They only dissected one specimen from north of California, where the spotted form would be common (Valdés & Gosliner, 2001), and this specimen was taken subtidally, where the ring form is most common. One of their pictured examples does fit the spotted pattern (Fig. 2E; Behrens & Valdés, 2001) but was not among the specimens dissected. Another of their specimens (Fig. 2F; Behrens & Valdés, 2001) is not clearly a spotted form or leopard form. A survey of photos available on the internet reveals several other ambiguous individuals (Fig. 1C), suggesting characterization of morphs is incomplete.

These differences in color, habitat, size and chemistry could also arise by intraspecific variation, but there are enough correlated differences between the morphs that mating or genetic studies should be used to test whether these represent separate species. Likewise, *Cadlina laevis* (Linnaeus, 1767) may also be two species specialized to different depths and prey (Rudman & Bergquist, 2007). Such cryptic species complexes of nudibranchs may prove a fruitful area for research.

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Shane Servant, James Mortimor and the Bamfield Marine Sciences Centre divers helped to collect specimens. Marli Wakeling and Jackie Hildering helped me track down references to the “leopard dorid.” Comments from Bill Rudman and Ángel Valdés greatly improved this manuscript. Financial support came from National Science and Engineering Research Council of Canada operating grant A7245-06395 to A.R. Palmer and a sabbatical leave to BKP from Saint Anselm College. To all I am extremely grateful.

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LETTER TO THE EDITOR

LITERATURE CITATIONS:
AUTHORS OF TAXA WITH DATE OF PUBLICATION SHOULD BE MANDATORY

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In *Malacologia* 55(1), there is an article by Arrighetti et al. (2012) on the growth of *Oliv-ancillaria deshayesiana*¹. The author of that taxon, and the date of its publication, appear nowhere within the article. Investigation reveals that the author is Ducros de Saint Germain, who described the species in 1857 in the genus *Oliva*. This author’s work is not in the Literature Cited. If the authors did not consult Ducros, they should at least have given an indication of the origin of the name. Two of the coauthors published a paper in 2011 in which this species was properly attributed to Ducros, so it is not for lack of knowledge that this information was omitted. Readers of this, or any, paper should not have to do a literature search to determine the author and date of a taxon if they wish to determine exactly what species is being treated. To their credit, the authors did include a photograph of the species they treated.

Other binomina appear throughout the paper, none of which have author and date appended. It is my opinion that not only should such data be mandatory but that there should be accompanying citations to the original sources. If a work cannot be consulted, which is not a rare occurrence, and identification is based on the work of others, the original work should still be cited and noted in the Literature Cited as “[not seen]”. This allows anyone interested to go to the original source of the name and also indicates that the present authors are familiar with the origin of the name. (Use of the term “not seen” does not detract from a citation, but it rather adds to the credibility of the authors.)

Also, I am obliged to file what I consider another important complaint. Within the text of the article under discussion is a statement of comparison made in the form “as in *O. auricularia* (Rocha-Barreira, 2003).” This is, of course, the form for citation of a binomen complete with author and date. However, *Olivancillaria auricularia* was described by Lamarck in 1810 in the

genus *Oliva* and not by Rocha-Barreira in 2003, who cited the species correctly. Authors should be requested to avoid this incorrect form of reference and distinguish the name of a taxon from references to it by later authors.

If requiring that all taxa referenced have original author and date, with an accompanying reference in Literature Cited, does not fit within the editorial mandates of *Malacologia*, it is requested that it be added to the list of recommendations for each author.

As an original subscriber to, and supporter of, *Malacologia* I was hesitant to write these comments which are hopefully constructive.

Respectfully submitted, Richard E. Petit

RESPONSE BY THE EDITORS

We agree that more uniform citation of the authors of taxa discussed in articles published in *Malacologia* would be useful, and we will modify our instructions to authors to require this. The new recommendation would be that all papers mentioning molluscan species should cite their authors and dates in proper form. If a paper concerns or mentions only a single species or only a few species, including anatomical, physiological, or ecological studies, the Literature Cited should include citations of the original descriptions of these taxa. For those species only listed as part of a faunal or ecological study and not otherwise discussed, citations of the original descriptions are not required.

It is already the policy of *Malacologia* that the text of articles should not include text citations of papers in a way that these can be confused with the authorship of taxa, and the copy editor often rewords sentences to ensure that this is the case. In the instance cited by Petit above, we missed one such needed modification.

¹F. Arrighetti, V. Teso, T. Brey, A. Mackensen & P. E. Penchaszadeh, 2012, Age and growth of *Olivancillaria deshayesiana* (Gastropoda: Olividae) in the southwestern Atlantic Ocean. *Malacologia*, 55(1): 163–170.

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